I. PRODRUGS BASED ON THE SULFAMATE LINKAGE

II. SELENINIC ACIDS AS BIOMIMETIC ENZYME INHIBITORS

by

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and approved by

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ABSTRACT OF THE THESIS

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Thesis Director: Prof. Spencer Knapp

The most important two features of a successful drug are the biochemical effects of active pharmaceutical ingredients and the efficacy of their delivery methods. Synthetic organic chemistry plays crucial roles in virtually both of these aspects for the design and the development of new drugs. Herein, in the first half part of the dissertation, a novel synthesis strategy of sulfamate esters is developed, which has been successfully applied into the synthesis of several tripartate pro-drugs with the purpose of improving the delivery efficiency of ethinyl estradiol. Their pharmaceutical effects are evaluated by both *in vitro* and *in vivo* studies as described. In the second half part, novel synthesis of several biomimetic enzyme inhibitors, including selenoxide, diselenide, seleninic acid, and various other organoselenium compounds is reported. Evaluation of these biomimetics will be elucidated in the future.

Dedication

To my loving parents Xiaoqin Li and Zhiyong Sun.

Acknowledgement

Reminiscing upon the times of research that I have dedicated to produce this dissertation, I would like to express my gratitude sincerely to many people that have poured their spirits on smoothing the challenging path that I have experienced.

Firstly, I would like to show the greatest respect to my supervisor, my mentor, and the biggest thinker that I have ever met in my life: Prof. Spencer Knapp, for your guidance, support, and encouragement throughout my thesis studies. The three years research experience in your group was a wonderful journey for my personal growth, both in knowledge and in intellect. Despite many obstacles we have met during the exploration of the scientific world, it is the wisdom and perseverance that you have been bestowed upon me during these research projects that leads me towards the final success.

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My gratitude goes to other people in the department, especially to the fellows in "Knapp's group", who have rendered me their support and advice. And my love also goes to my family. My parents Xiaoqin and Zhiyong have been with me every step of the way with their constant hope, encouragement and wisdom; I am proud to be their son. And this dissertation cannot be achieved without their earnestly inculcation.

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List of Abbreviations

BF_3	boron trifluoride
BOC	*tert*-butyloxycarbonyl
CYP3A4	cytochrome P450 3A4
CYP2C9	cytochrome P450 2C9
CYP2C19	cytochrome P450 2C19
CYP2D6	cytochrome P450 2D6
CYP1A2	cytochrome P450 1A2
Cys	cysteine
DCM	dichloromethane
DMDO	dimethyldioxirane
DMSO	dimethyl sulfoxide
EE	ethinyl estradiol
hERGs	human ether-*a*-*go*-*go*-related gene
KSeCN	potassium selenocyanate
LDA	lithium diisopropylamide
m-CPBA	*meta*-chloroperoxybenzoic acid
ODCase	orotidine monophosphate decarboylase
OMP	orotidine 5'-monophoaphate
OPRT	orotate phosphoribosyltransferase
PBS	phosphate buffered saline
TBDMS	*tert*-butyldimethylsilyl
t-$BuNO_2$	*tert*-butyl nitrite
THF	tetrahydrofuran

TLC	thin layer chromatography
UMP	uridine 5’-monophosphate

Chapter 1. Prodrugs Based on the Sulfamate Bond Linkage System

1.1. Background

1.1.1. The quest for pro-drugs

The continual development of new drug delivery systems is driven by the needs to maximize therapeutic activities while minimizing negative side effects.[1] With the application of modern discovery techniques like high-throughput screening, novel lead structures with high pharmacological potency can be efficiently discovered. However, there are several obstacles to drug development that affect patients' compliance: chemical instability, low aqueous solubility, inadequate brain penetration, insufficient oral absorption, or unpleasant odor and taste.[2] The necessity for minimizing the risk of such properties has lead to the establishment of pro-drugs as a means to improve the biopharmaceutical, pharmacokinetic and pharmacodynamic aspects of the active ingredients.[3]

The term pro-drug was first introduced in 1958 by Adrien Albert to describe compounds that undergo biotransformations prior to eliciting their pharmaceutical effects.[4] Although pro-drug techniques have been in use for a couple of decades, they have recently been growing in popularity. Pro-drugs, often referred to latent drugs or bioavailable drug-carrier conjugates,[5] are inactive derivatives.[6] To synthetic chemists, pro-drugs can be thought of as molecules that bear biochemically labile protecting groups. In general, pro-drugs can be categorized as carrier-linker pro-drugs, bioprecursors, site-specific chemical delivery systems, macromolecules, and drug-antibody conjugates.[3,7]

1.1.2. The function and limits of ethinyl estradiol

Ethynyl estradiol (EE, Figure 1.1) is the first known orally-available synthetic steroidal estrogen, and has been used for a wide variety of conditions and purposes. This compound is in almost all modern formulations of combined oral contraceptive pills, with pregnancy prevention as the chief function. Substitution at C-17 of the estradiol steroid with an ethinyl group has proven to retard biodegradation and this change has paved the way for the oral delivery.[8] Despite its successful application in birth control for decades, ethinyl estradiol still suffers from a short half-life and rapid deactivation *in vivo*. After absorption in the small intestine, ethinyl estradiol undergoes extensive metabolism in the liver involving oxidation and hydroxylation by cytochrome P450 3A4 isoenzymes. Other types of metabolism include the conjugate transformation of estrogen hormones into less active and more water-soluble metabolites that facilitate their excretion in bile and urine.[9-11] For those reasons, EE usually requires daily administration to be effective, albeit the majority of the drug loses activity rapidly after being orally administered. Therefore, it is desirable to design a delivery system to elongate the *in vivo* half-life of ethinyl estradiol for the purposes of its efficiency improvement.

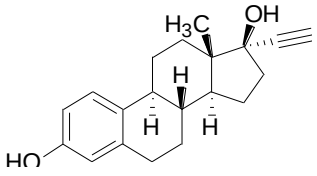


Figure 1.1. Ethinyl estradiol

1.1.3. Sulfamate ester as a neo-linker

In the past decade, there has been a surge of interest in sulfamate esters, due to their potential ability to block a variety of enzymatic pathways.[12,13] The hydrolysis mechanism of sulfamate esters under different pH conditions has been elucidated.[14,15] According to the research by Spillane et al., the two principal elimination pathways are E1cB and E2 depending upon the environment's pH values. As shown in Figure 1.2, ester can initially deprotonate once or twice to form the anion, followed by a rate-determining rupture of the ArO-S bond. The newly formed N-sulfonylamide or the anion is rapidly attacked by solvent molecules to yield sulfamic acid or aryl oxide products. However, recent studies show that an alternative associative S_N2 mechanism[14] occurs at moderate acidity where water acts as a nucleophile to attack the sulfur atom so the sulfamic acid and phenol are produced with a relatively slower hydrolysis rate.

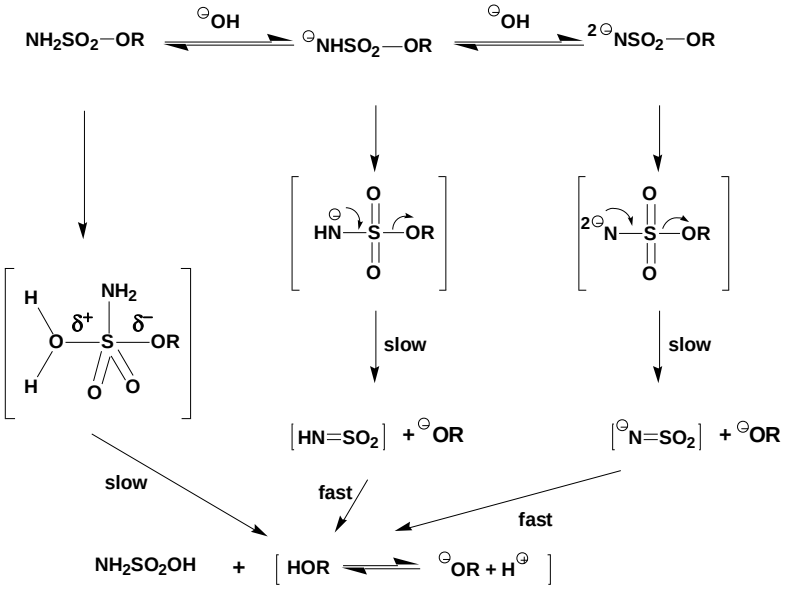
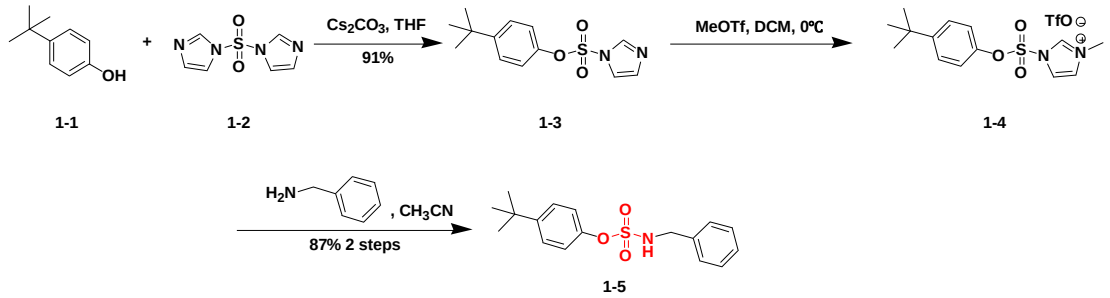


Figure 1.2. Proposed sulfamate hydrolysis pathways in different pH environments[15]

1.2. Synthesis of sulfamate conjugates

1.2.1. Model reactions for sulfamate ester synthesis

The synthesis of the sulfamate bond was modeled in a three-step route using 4-*tert*-butylphenol and benzylamine as test reagents. Sulfamate **1-3** was prepared from **4**-*tert*-butylphenol and 1,1'-sulfonyldiimidazole.[16] The subsequent N-methylation on the imidazole ring of **1-3** provided triflate salt **1-4**, which was then transformed into the sulfamate ester by the substitution of the primary amine.[17] In three steps, the sulfamate ester was synthesized with an 80% overall yield in mild conditions.

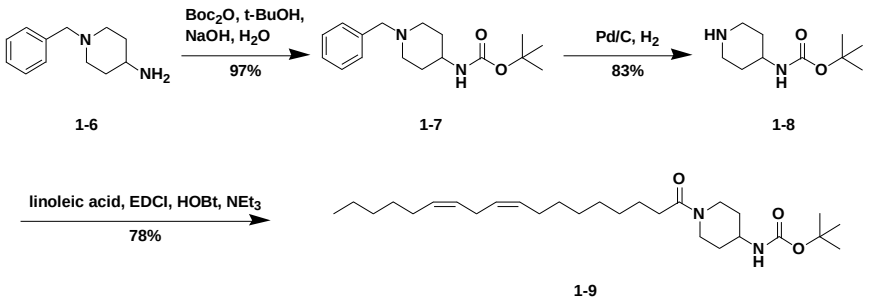


Scheme 1.1. Model reactions for sulfamate ester synthesis

1.2.2. Synthesis of the carrier moiety

Linoleic acid has been selected as a carrier candidate of the pro-drug conjugate. The synthesis was initiated by the protection of the primary amine **1-6** with the BOC group; subsequent hydrogenolysis to release the benzyl group afforded the secondary

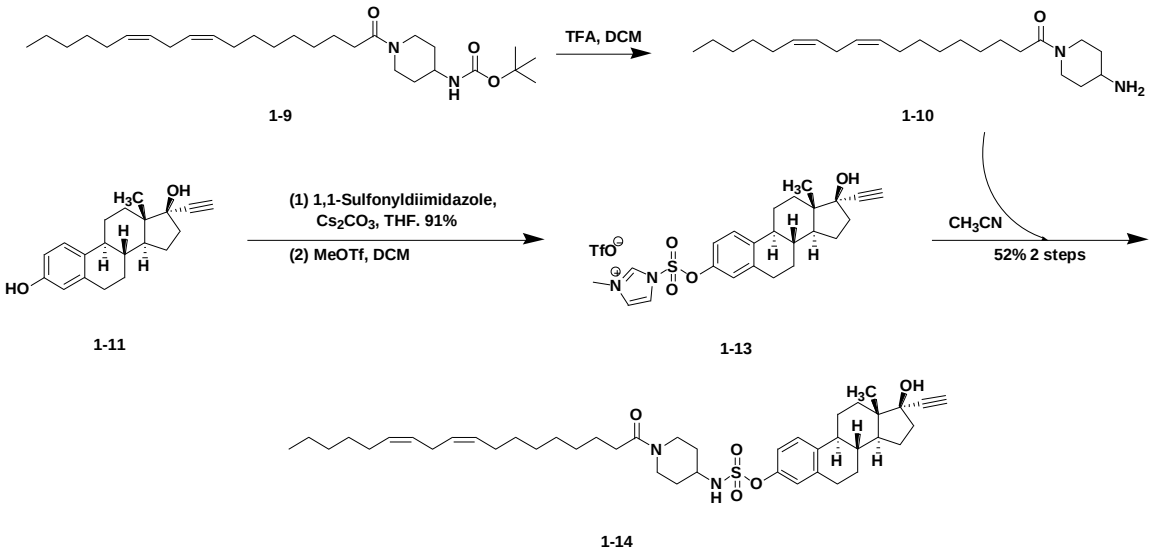
amine **1-8**; standard amidation of **1-8** with linoleic acid provided amide **1-9** as the carrier portion.[18]



Scheme 1.2. Synthesis of carrier moiety of sulfamate conjugate

1.2.3. Synthesis of the linoleoyl pro-drug conjugate

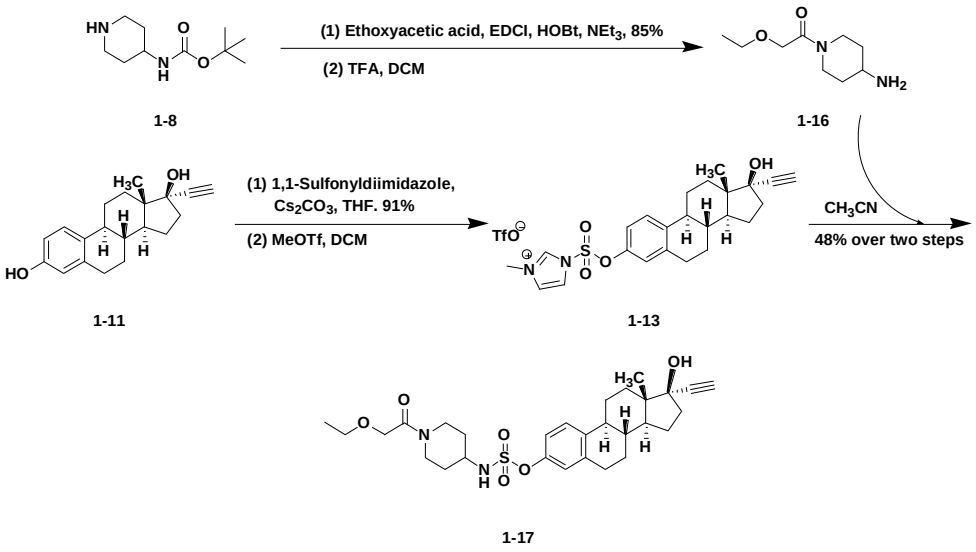
The conjugate carrier **1-10** was achieved by treating **1-9** with 40 % trifluoroacetic acid in DCM solution. Ethinyl estradiol was mixed with 1,1-sulfonyldimidazole under basic conditions, followed by the methylation in dichloromethane to afford the triflate salt **1-12**. The pro-drug precursor **1-13** was then coupled with the carrier **1-10** to form the final product **1-14** in a decent yield. The reaction was performed under the same conditions as the model reaction, but gave lower yields. One of the challenges here is to avoid the decomposition of the unstable triflate salt **1-13**. Other undesired side reactions contributing to the lower yield include premature hydrolytic cleavage of the sulfamate ester bond and methylation on the C-17 hydroxyl group.



Scheme 1.3. Synthesis of the linoleoyl pro-drug conjugate

1.2.4. Synthesis of the ethoxyacetyl pro-drug conjugate

Given that the lipophilic linoleoyl group increases the hydrophobicity of the conjugate molecule and affects its absorption efficiency, ethoxyacetic acid was selected as a more polar carrier to improve the water solubility of the conjugates. The carrier **1-16** was synthesized by coupling piperidine **1-8** with ethoxyacetic acid and subsequent deprotection in aqueous TFA solution. The pro-drug precursor **1-13** was synthesized by following the same procedure. The coupling of **1-13** and **1-16** was performed under the same conditions to give the ethoxyacetyl pro-drug conjugate **1-17** in a comparable yield.



Scheme 1.4. Synthesis of the ethoxyacetyl pro-drug conjugate

1.3. *In vitro* studies of pro-drug conjugates

1.3.1 Hydrolysis performance of two conjugates at different pHs

An *in vitro* hydrolysis study of the two conjugates **1-14** and **1-17** has been carried out in a solution of physiological pH (7.4) buffer and ethanol at 37℃. The results indicate a half-life of approximately 7.5 days for both compounds. Both conjugates share the same mechanistic pathway of hydrolysis by cleavage of the S-O bond of the sulfamate ester to release ethinyl estradiol without the observation of any other steroid product. The comparable half-lives show that carrier components have little influence on the overall releasing kinetics. The major difference is that compound **1-15** takes less time to be completely dissolved in the aqueous buffer, resulting in a slightly different hydrolysis rate pattern for the first two days.

To further explore the hydrolysis mechanism, three parallel groups of conjugates **1-14** were hydrolyzed at pH 2.0, pH 5.0 and pH 7.4 under the same conditions. The results demonstrate that the hydrolysis rate of sulfamate ester is pH-independent within the range of pH 2.0 to 7.4 since their half-lives are all approximately 7.5 days. Such results eliminate the E1cb pathway from the hydrolysis mechanism and strongly support the passive hydrolysis of the sulfamate bond by nucleophilic attack of water on the sulfamate ester to release ethinyl estradiol directly.

1.3.2. Summary of *in vitro* study results

To further analyze the properties of sulfamate pro-drug conjugates, compound **1-14** was evaluated in a variety of *in vitro* media. Solution properties are summarized in Table 1.1 and the data supports its expected poor water solubility and high lipophilicity. Protein binding data was not measurable, but based on the strong binding affinity of ethinyl estradiol towards plasma albumin, we assumed that the conjugate completely binds to the protein and no free molecules remain in the plasma. The *in vitro* metabolism data encourages further investigation on these types of compounds since 46% of prodrug molecules survive the mechanism in the liver. But the compound also exhibits strong inhibition properties towards several important enzymes in liver including CYP3A4, CYP2C9 and CYP2C19. Such properties might lead to unfavorable drug-drug interactions and need to be addressed in the further research. Cardiac toxicity results are promising, since a relatively high concentration of conjugates can be tolerated without hERG inhibiton.

Table 1.1. Summary of linoleoyl pro-drug conjugate *in vitro* properties

Compound	Properties	Test Concentration (M)	Results	
1-14	Aquesous Solubility (PBS, pH 7.4)	2.0E-04	Mean (μM)	4.9
1-14	Partition Coefficient (log D, n-Octanol/PBS, pH 7.4)	1.0E-04	Weighted Average of Three Replicate	> 3.9
1-14	Protein Binding (Plasma, human)	1.0E-05	Flags	Not Detected
1-14	Metabolic Stability (liver microsomes, human)	1.0E-06	Mean Parent Remaining (%)	46
1-14	CYP1A2 Inhibition (recombinant, CEC substrate)	1.0E-05	% Inhibition of Control Values	31
	CYP2C9 Inhibition (recombinant, MFC substrate)	1.0E-05		80
	CYP2C19 Inhibition (recombinant, CEC substrate)	1.0E-05		69
	CYP2D6 Inhibition (recombinant, MFC substrate)	1.0E-05		44
	CYP3A4 Inhibition (recombinant, BFC substrate)	1.0E-05		100
1-14	hERGs (automated patch-clamp)	1.0E-07	% Inhibition of Tail Current	0.5
		1.0E-06		-1.0
		1.0E-05		13.9

1.4. *In vivo* biological evaluation of pro-drug conjugates

Conjugate **1-14** was formulated in an encapsulated solutol solution and tested in beagles via oral delivery. The results of this study are summarized on Table 1.2. Successful release of ethinyl estradiol from the conjugate molecules is discovered at an even slower rate compared with *in vitro* experiments. The pro-drug molecules are barely detected in the plasma due to its strong binding affinity towards albumins and poor solubility. The release of the linoleamide is delayed for a period of 4 to 6 h. But the concentration quickly reaches its maximum value at 8 h, and then decreases till the end.

In conclusion, the slow release of ethinyl estrodiol from parent drug molecule is achieved, and we are now working on other conjugate systems with novel carriers and linkers to improve the drug *in vivo* performance.

Table 1.2. *In vivo* canine tests results of pro-drug conjugates

	Dog 1			Dog 2		
Time Point	EE	EE-SULF	Linoleamide	EE	EE-SULF	Linoleamide
(h)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
0.25	0.0212	BLQ	BLQ	0.0106	BLQ	BLQ
0.5	0.0355	BLQ	BLQ	BLQ	BLQ	BLQ
1	0.0251	0.189	BLQ	BLQ	BLQ	BLQ
2	0.0287	0.661	0.00839	BLQ	BLQ	BLQ
4	BLQ	0.172	0.0177	0.0152	BLQ	BLQ
6	0.0263	0.0601*	0.022	BLQ	BLQ	0.00231
8	0.0339	0.00647*	0.0305	0.00968	BLQ	0.00456
24	0.025	BLQ	0.0282	0.0131	0.242	0.0184
48	0.0228	BLQ	0.0244	0.0211	BLQ	0.0263
54	0.0226	BLQ	0.0134	0.015	BLQ	0.0163
72	0.0122	BLQ	0.0123	0.0193	BLQ	0.0197
78	0.00897	BLQ	0.00549	0.0109	BLQ	0.0129
96	0.0113	BLQ	0.000857	BLQ	BLQ	0.0199

1. BLQ: Below Limit of Quantitation, BLQ<0.050 ng/mL for EE, BLQ<0.010 ng/mL for EE-SULF, BLQ<0.010 ng/mL for Linoamide.

2. *: Extrapolated value.

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Chapter 2. Aliphatic Seleninic Acids as Potent Cysteine Protease Inhibitors

2.1. Biomimetic enzyme inhibitors

In biomimetic chemical research, the purposeful synthesis of unnatural compounds that mimic the structures and the functions of compounds with biological activities is a major task. Despite the high affinity for natural substrates in bio-systems, such compounds still possess undesirable aspects of physicochemical properties like strong toxicity, poor solubility, metabolic instability, and unfavorable selectivity. Therefore, artificially synthesized mimics represent a possible avenue to compounds with improved features.

Bioisosterism is among the most commonly used strategies by medicinal chemists for the rational modification of undesirable characteristics in natural substrates.[1,2] This topic has been emphasized for many years. Its core concept is to modify the enzyme substrates with functional groups comparable in size, shape, electronic property, lipophilicity, and solubility.[2] The resulting compounds thus retain many of the desirable properties of the original compounds while minimizing or eliminating undesirable ones.

2.2. Fortuitous binding inhibitors for cysteine proteases

2.2.1. Cysteine protease and their inhibitors

Proteases and proteolytic enzymes form one of the largest and more important groups of enzymes. The large families of peptide-bond cleavage enzymes can be categorized into 4 major classes based on their characteristic groups at the active sites:

serine, aspartic, cysteine and metalloproteases.[3] Cysteine proteases are enzymes that degrade polypeptides with a common catalytic mechanism that involves a cysteine thiol in the catalytic cycle. Cysteine proteases can be found in viruses,[4] bacteria,[5] fungi,[6] plants,[7] mammals, and humans.[8] They are involved in protein coding, periodontal disease, parasite nutrition, host cells invasion, blood coagulation, and fertilization. Uncontrolled, unregulated, or undesired cysteine proteases can lead to many diseases states including emphysema, stroke, cancer, inflammation and arthritis. The inhibitors for this type of enzyme thus have considerable potential utility in the pharmaceutical intervention for a variety of diseases.

Hydrolysis of a peptide bond is an energetically favored reaction, but is normally extremely slow.[9] Although the molecular basis of the mechanism for the hydrolysis catalyzed by cysteine proteases is not completely elucidated, it is certain that the high catalytic capacity of cysteine proteases can be explained by the formation of a tetrahedral intermediate as shown in Figure 2.1. The thiolate ion firstly attacks the carbonyl group of the peptide bond to be cleaved, and a tetrahedral hemithioacetal is formed; an oxyanion hole created by the hydrogen bonds on the backbones of enzymes stabilizes the intermediate oxyanion. The subsequent cleavage of the peptide bond releases the N-terminus and a hydrolysis step restores the enzyme.[10-12]

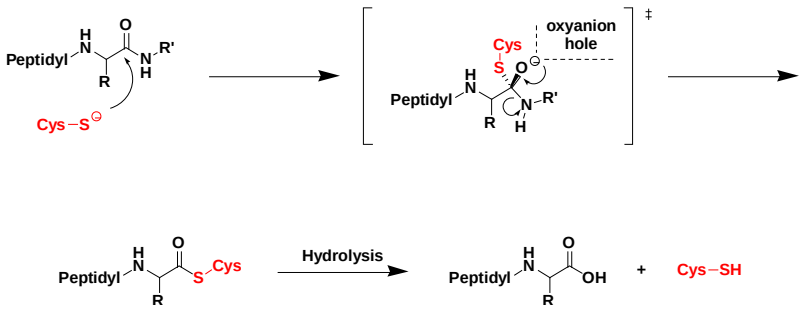


Figure 2.1. Mechanism of cysteine proteolysis

A wide variety of approaches have been utilized on the design of cysteine protease inhibitors. The most common strategy is the appropriate modification of the substrates by the attachment of a warhead targeting on the active sites of the enzymes. Based on the abovementioned catalytic mechanism, the warheads could either participate into the catalytic circle by mimicking the tetrahedral intermediates for the reversible inhibition (compound 1, 2, Figure 2.2)[13,14] or interact with the mercaptan covalently to irreversibly inhibit enzymes (compound 3 – 8, Figure 2.2).[15-20] Despite the continuing investigation on the development of inhibitors, few of them have been successfully converted into marketed drugs. Most of the candidates fail on the clinical trials due to deficiencies in reactivity, selectivity or bioavailability.[21] A pharmaceutically effective protease inhibitor should be strongly reactive with multiple enzymes, possess high selectivity and low toxicity, and achieve the efficient bioavailability for delivery. The quest for new protease inhibitors therefore deserves emphasis for the clinical treatment of various diseases.

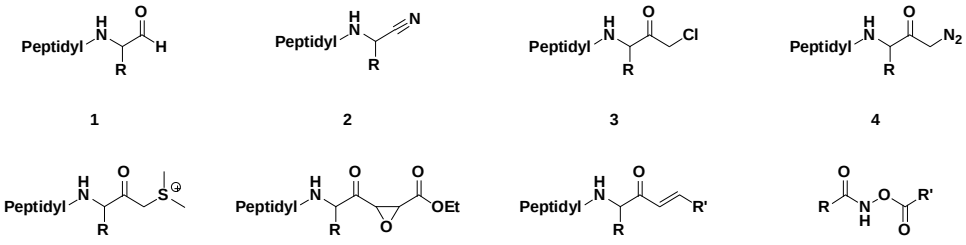


Figure 2.2. Cysteine protease inhibitors

2.2.2. Seleninic acid as bioisosteres

Biological anionic functionality such as *O*-phosphate, *O*-sulfate, and carboxylate exists prominently in nature, particularly in biological substrates that activate an assortment of catalytic cycles that directly or indirectly engage in the physical processes and in the etiology of disease states. For example, tetrazoles, amides and phosphonates have been developed as isosteres for the carboyxlates.[22] Organoselenium compounds have been found or induced in nature and have been studied in the laboratory in all four oxidation states: selenol, selenenic, seleninic, and selenonic.[23,24] They are important as reagents and intermediates in organic synthesis,[25] heavy-atom versions of oligonucleotides, human metabolites, cancer-preventative agents,[26] and as potential substrates for biomimetic studies.

Seleninic acids resemble carboxylic acids in structure by a selenium atom substituting the carbon atom of the carbonyl group. They are unique in that they can be either protonated or deprotonated[27] at physiological pH and can accept Lewis bases at selenium to form tetracoordinate trigonal bipyramidal adducts.[28] With the similar size, charge and pKa values, seleninic acid has been advanced as bioisosteres for carboxylic acid and its derivatives including ester and amide. Furthermore, seleninic acids can couple with thiols over a wide pH range to give a redox product, the mixed selenosulfide. The details of these reactions have been established in several authoritative papers by Kice and Knapp.[29,30] The initial condensation of seleninic acids with thiols produce thioseleninate and water quickly and completely. The subsequent isomerizations of thioseleninates generate a series of coupled and *S*-oxidized products. The redox reaction

thus demonstrates the feasibility of coupling with the Cys residue in the active cites of cysteine proteases or other enzymes containing thiol groups in the active sites. A new type of peptidyl seleninic acid (Figure 2.3) as a potential cysteine protease inhibitor is designed based on this concept. Effective irreversible enzyme inhibitors possibly can be found and serve as potent drugs candidates.

Figure 2.3. Seleninic acid derived cysteine proteases inhibitors

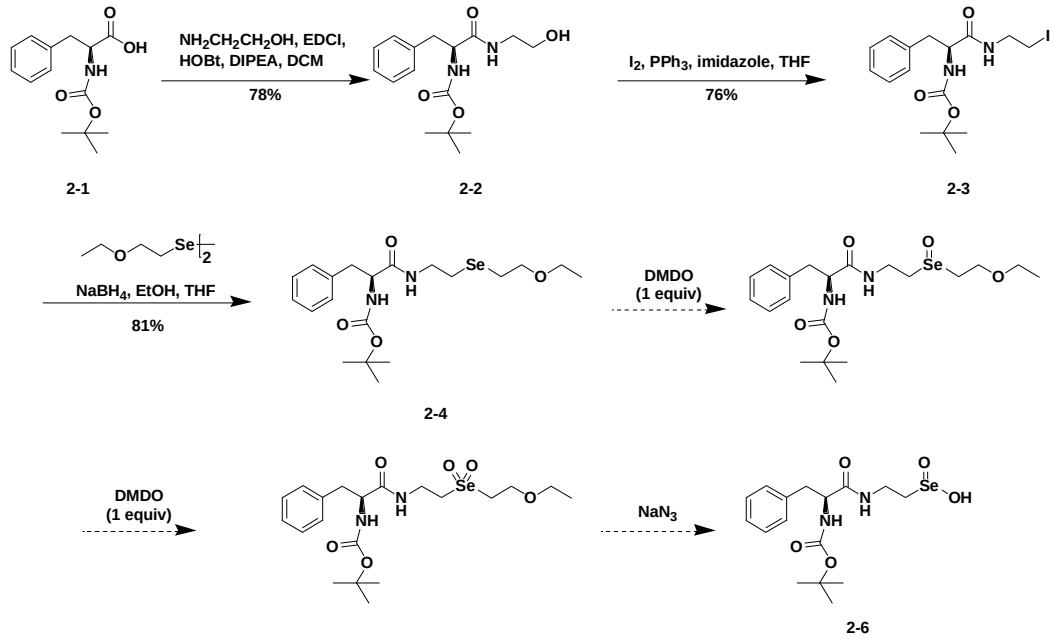
2.3. Synthesis of peptidyl seleninic acids

2.3.1. Attempted synthesis of seleninic acid via oxidation of selenide

The first synthetic route to the seleninic acid proceeds by the dealkylation of selenones with sodium azide. The selenones can be generated by the oxidation of selenoethers with an excess of strong oxidant such as dimethyldioxirane (DMDO) or *meta*-chloroperbenzoic acid (m-CPBA).[31,32]

As shown in Scheme 2.1, the standard amidation of Boc-L-Phe with 2-aminoethanol produced amide **2-2** with a good yield. Subsequent substitution of the hydroxyl group of **2-2** by the iodine generated iodide **2-3**. Selenide **2-4** was obtained after the addition of iodide **2-3** into a premixed solution of bis(ethoxyethyl) diselenide and sodium borohydride. However, oxidation of **2-4** was plagued by side reactions, including the presume *syn* β-elimination of the selenoxide to form the N-vinylamide. The 2D TLC

analysis indicated that a presumably oxidative product formed quickly after the addition of the oxidant and then it gradually decomposed on the TLC plate. Due to the difficulty of isolating the unstable selenoxide, we abandoned this attempt and devised alternate synthesis routes.

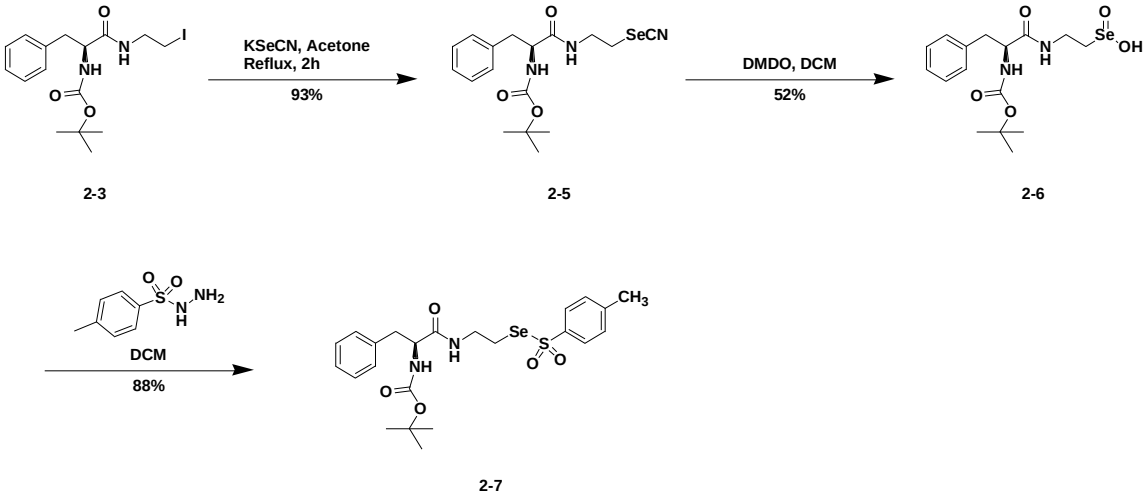


Scheme 2.1. Attempted synthesis via selenoether

2.3.2. Synthesis of seleninic acid via selenocyanates.

Many seleninic acids have been prepared by the oxidation of selenocyanates with strong oxidants like concentrated hydroperoxide, nitric acid, or peracetic acid.[33] Aliphatic selenocyanates, R-SeCN, can be easily prepared when potassium selenocyanates and alkyl chlorides or alkyl bromides are heated in polar solvents like acetone or methanol. Based on this strategy, the second generation of synthesis approach to the seleninic acid

was designed as shown on Scheme 2.2. The selenocyanate **2-5** was obtained in excellent yield by the stirring of iodide **2-4** with potassium selenocyanate in refluxing acetone. The oxidation of **2-5** with DMDO produced the seleninic acid **2-6** with a decent yield. The structure of seleninic acid **2-6** was examined by ^{1}H, ^{13}C, ^{77}Se NMR spectrum and MS spectrum. Its validity is further supported by the redox coupling of the seleninic acid with *p*-toluenesulfonylhydrazide to give selenosulfonate **2-7**.

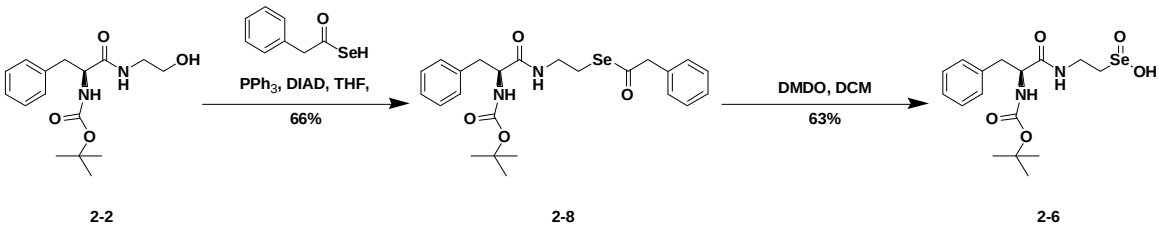


Scheme 2.2. Synthesis of seleninic acid via selenocyanate

2.3.3. Synthesis of seleninic acid via selenoester

Selenoesters have been widely applied in the synthesis of seleninic acids, and the reactions usually have high yields. An exceptional work to produce selenoesters from the novel selenocarboxylic acids has been illustrated in two authoritative papers by Knapp.[34,35] Therefore, an alternative route for the synthesis of seleninic acid **2-6** was attempted (Scheme 2.3). To abridge the synthesis route, selenoester **2-8** was prepared by

a selenocarboxylate Mitsunobu conversion from amide **2-2**. The subsequent oxidation produced seleninic acid **2-6** in fair yield.



Scheme 2.3. Synthesis of seleninic acid via selenoester

Two good synthetic routes for the peptidyl seleninic acids have been designed. Future work will extend this methodology to other amino acid substrates. To obtain oligopeptides derived seleninic acids, the peptidyl components of the molecules should be synthesized first to minimize side reactions. The biological evaluation of **2-6** and its analogs as potential inhibitors of cysteine proteinases will be investigated in the future.

2.4. References

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Chapter 3. Uridine Derived Seleninic Acid as an Inhibitor for Orotidine Monophosphate Decarboxylase (ODCase)

3.1. Orotidine monophosphate decarboxylase (ODCase)

Orotidine monophosphate decarboxylase (ODCase) catalyzes the decarboxylation of orotidine 5'-monophosphate as shown in Figure 3.1.[1,2] In yeast and bacteria, ODCase occurs as a protein that appears to have this single function.[3,4] While in mammals, the enzyme constitutes part of a bifunctional enzyme named UMP synthase that in addition catalyzes the preceding reaction in pyrimidine nucleotide biosynthesis.[5,6] The enzymatic conversion of orotidine 5'-monophosphate (OMP) to uridine 5'-monophosphate (UMP) is the final step of *de novo* pyrimidine nucleotide biosynthesis.[7] The non-enzymatic reaction has been described as the slowest biological reaction with a half-life of 78 million years. ODCase accelerates the reaction by a factor of 1.4×10^{14} without the participation of any cofactors or metal ions, making it a very proficient enzyme.[5,8]

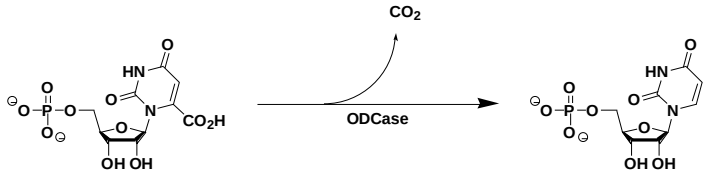


Figure 3.1. The enzymatic decarboxylation of OMP to UMP

The remarkable catalytic ability of ODCase has attracted significant attention on its catalytic mechanism.[2,4,8,9] Recent crystallographic studies indicate the presence of a

polar environment (salt-like) of the active-site region (See Figure 3.2).[10,11] Although the veritable mechanism of this catalytic cycle still remains as a mystery, it is recognized that the driving force for the loss of the carboxyl group linked to the C6 of pyrimidine ring comes from the close proximity of an aspartate residue carboxyl group in the enzyme's active site. The strong electronic repulsion between the two anions destabilizes the ground state relative to the transition state of the uncatalyzed reaction.[12,13]

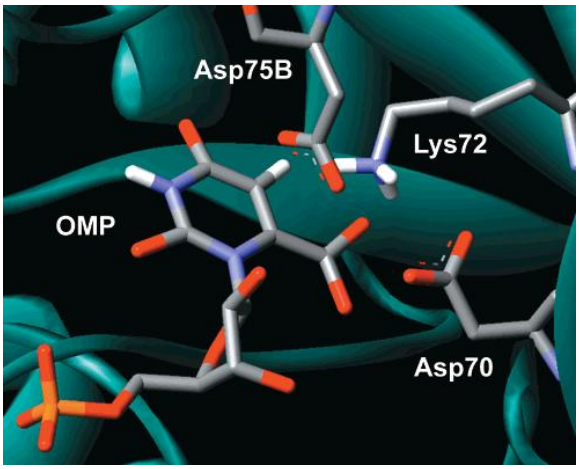


Figure 3.2. Active-site region of ODCase

3.2. Inhibitors for ODCase

Regarding the significant importance of ODCase in nucleotide synthesis and regulation, ODCase inhibitors could potentially exhibit a variety of biological activities including antiviral, antiplasmodial, and anticancer activities.[14] Several 5'-monophosphate analogues of classic nucleotide derivatives, such as 6-hydroxyuridine,[1] 6-

thiocarboxamidouridine,[15] 6-azauridine,[16] pyrazofurin,[17] are potent inhibitors of ODCase (Figure. 3.3). Since the discovery of this type of compounds as effective antimalarial agents, there has been a rise in interests to explore modified nucleotides as potent ODCase inhibitors.[18-20] Our previous lab workers have illustrated the inhibition of orotate phosphoribosyltransferase (OPRT),[21] an essential enzyme required for the *de novo* biosynthesis of pyrimidine nucleotide, by several organoselenium nucleotides and nucleosides. The further application of this novel type of nucleotide inhibitors on other nucleotides like ODCase will promote the development of new selenium chemistry and improve the knowledge of the catalysis mechanisms.

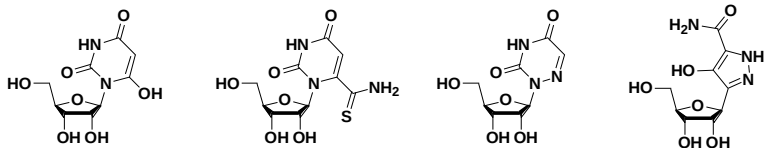


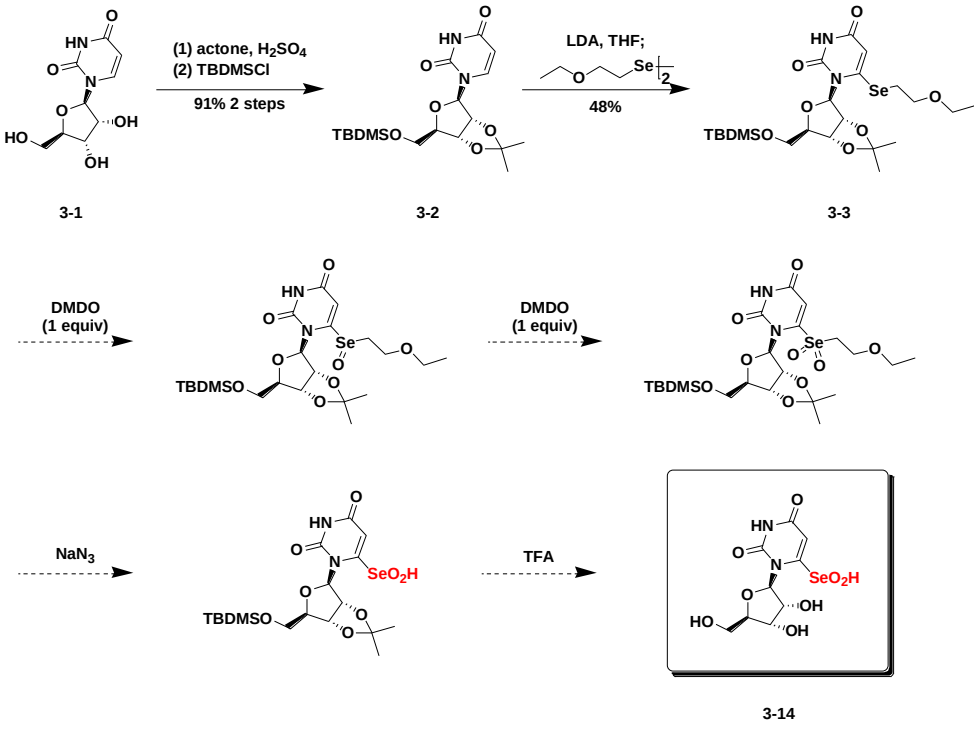
Figure 3.3. Structures of some ODCase inhibitors

3.3. Synthesis of 6-uridine seleninic acid

3.3.1. Attempted synthesis via selenones

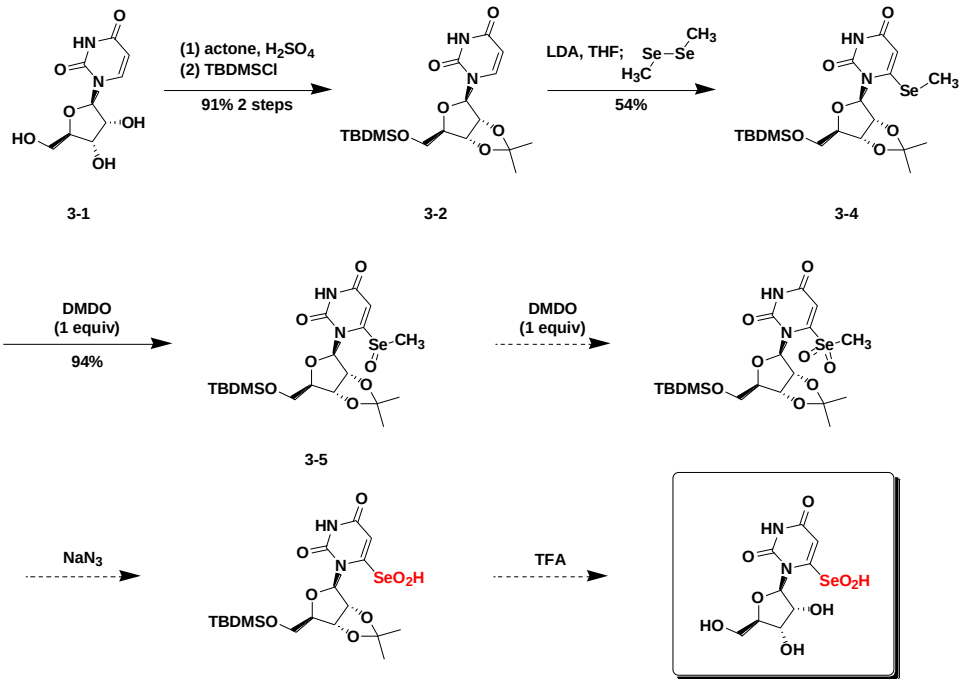
A uridine derived seleninic acid **3-14** was designed for the potential inhibition of the enzyme with the structure mimicing the substrate of ODCase. The first attempted synthesis was illustrated in the scheme 3.1. Protection of the triols of uridine proceeded in two steps with acetone and TBDMS chloride in excellent yields. After the lithiation by LDA/THF, electrophilic aromatic selenylation of uridine **3-2** with bis(ethoxyethyl)

diselenide generated selenide **3-3**. Unfortunately, the subsequent oxidation of selenide **3-3** was hampered by the labile oxidative product. Based on the 2-D TLC analysis, a selenoxide product was presumably formed within minutes after the addition of DMDO but quickly decomposed afterwards on the silica gel plates. One of the possible side reactions would be the *syn* β-elimination of selenoxide, leading to a formation of the selenenic acid or other more stable compounds. Decreasing the reaction temperature did not assist the isolation of the unstable intermediate, nor can it be further oxidized into selenone in presence of a excess of the oxidant for longer time.



Scheme 3.1. Attempted synthesis via bis(ethoxyethyl) diselenide

The best solution to eliminate the *syn* β-elimination is to exclude the β-H. Therefore, dimethyl diselenide was selected as the selenylation reagent. As shown in scheme 3.2, selenide **3-4** was synthesized in a parallel reaction with a decent yield. Subsequent oxidation by DMDO successfully transformed **3-4** into selenoxide **3-5**, which is stable for isolation and characterization by NMR spectroscopy and mass spectroscopy. However, further oxidation by DMDO failed as the selenoxide **3-5** remained in the solution after 48h at room temperature. One of the selenoxide diastereomer precipitated out of the solution gradually and it cannot be dissolved by most organic solvents except DMSO. The other diastereomer was reluctant to be oxidized due to the more hindered configuration. Increasing the temperature of the system or introducing other strong oxidants like m-CPBA or hydrogen peroxide failed as well to overcome the oxidation barrier of the selenone transformation.



Scheme 3.2. Attempted synthesis via dimethyl diselenide

3.3.2. Attempted synthesis via diselenides

Many aromatic seleninic acids are prepared by the oxidation of diselenides with hydrogen peroxide, potassium permanganate in acetic acid, chlorine in aqueous medium and ammonium peroxydisulfate (Figure 3.4). Some aromatic lithium compounds are reported in the literature to react with elemental selenium.[22] Intermediate R-Se-Li are expected to be formed and transformed into selenols (R-SeH) by subsequent hydrolysis. In most cases, the selenols are not isolated but may be easily oxidized by air or other oxidants to the diselenides or other more stable products. To utilize this strategy, uridine **3-2** was lithiated with LDA in the presence of elemental selenium. However, no exciting transformation was discovered and almost 100% starting material was recovered.

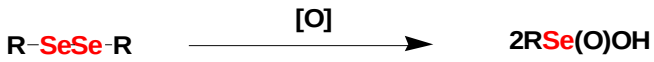
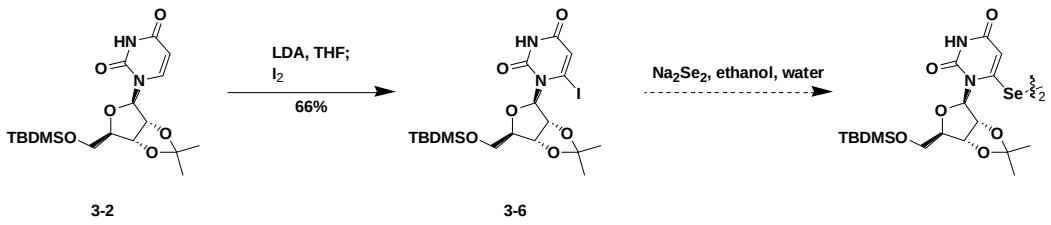


Figure 3.4. Oxidation of diselenide produces seleninic acid

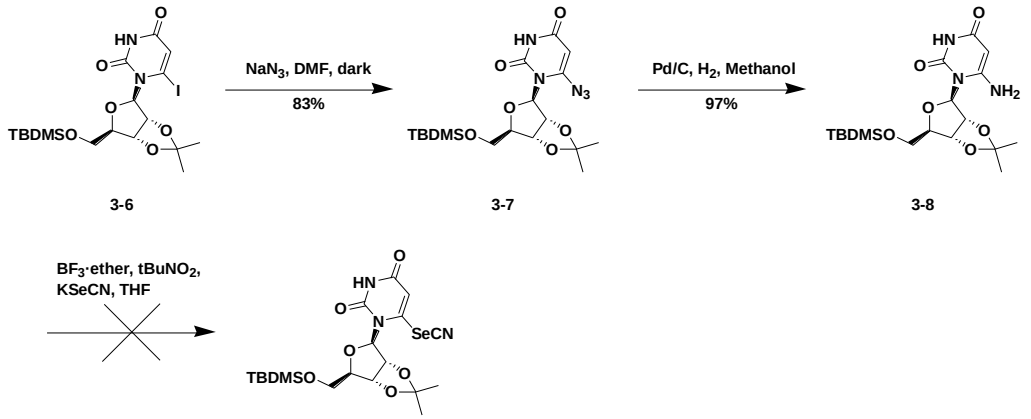
Diselenides can also be prepared by treatment of alkyl halides with disodium diselenide. For instance, bis(ethoxyethyl) diselenide is synthesized via the addition of 2-bromoethyl ethyl ether into a premade disodium diselenide aqueous solution.[21] To fulfill this concept, iodouridine **3-3** was synthesized with a decent yield as shown in Scheme 3.3. The second step, however, failed to inspire further exploring as the starting martial remained unaffected for many hours.



Scheme 3.3. Attempted synthesis via disodium siselenide

3.3.3. Attempted synthesis via selenocyanate

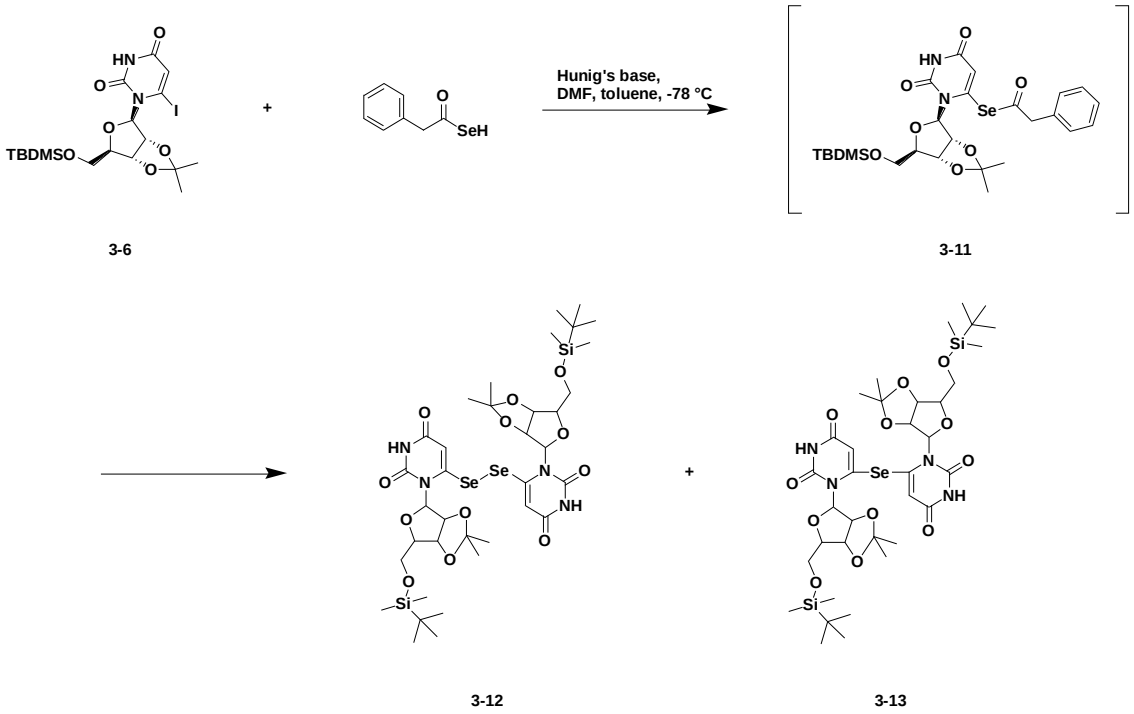
Selenocyanates, as seleninic acid precursors, have been widely used for the synthesis of organoselenium compounds. Unlike aliphatic selenocyanates, aromatic selenocyanates are generally not obtainable from halides and KSeCN unless additional substitutions in the aromatic ring enhance the reactivity of the halogen atoms.[23] Aromatic diazonium salts therefore serve as the major substrates for the preparation of aromatic selenocyanates. To prepare the uridine derived selenocyanate, iodouridine **3-6** was transformed into 6-aminouridine **3-8** in two steps via nucleophilic substitution and hydrogenation in excellent yields. Regarding the liability of the uridine moiety, mild reagents BF_3 ether and t-$BuNO_2$ were screened out for the diazoniation of **3-8**. Two products instead of the uridine derived selenocyanate were isolated beyond our expectation. They were characterized presumably as 5-carboxyuridine and a cyclic selenenate ester based on structure analysis. The mechanism for the formation of such compounds is still under investigation.



Scheme 3.4. Attempted synthesis via selenocyanate

3.3.4. Attempted synthesis via selenoesters

Oxidative cleavage of selenoesters generally produces seleninic acids in good yields. The outstanding work to obtain selenoesters from selenocarboxylates has been established in several authoritative paper by Knapp.[24,25] Treatment of iodouridine **3-6** with (2-phenyl)-selenoacetic acid produced a uridine derived selenoester **3-11** that was not isolable, but instead eliminated the benzyl acetyl group within minutes to give the presumed 6-uridine selenol intermediate. Diselenide **3-12** was formed by the oxidation of the selenol with atmospheric oxygen. Another product monoselenide **3-13**, which possessed the same Rf value as **3-12** that made these two compounds inseparable, was generated presumably by the substitution of the iodide of the iodouridine **3-6** by the selenonate anion (Scheme 3.5).



Scheme 3.5. Attempted synthesis via selenoesters

One more oxidation step will transfer the diselenide **3-12** into the seleninic acid. Unfortunately, the reaction of **3-6** with selenocarboxylate acids was plagued, giving **3-12** and **3-13** in only 20 % yield in total. We are currently working on the optimization of this route to obtain enough material for the further application.

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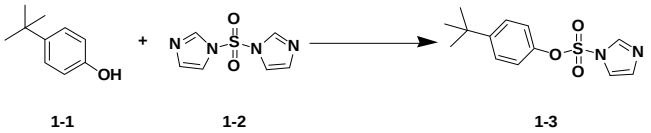
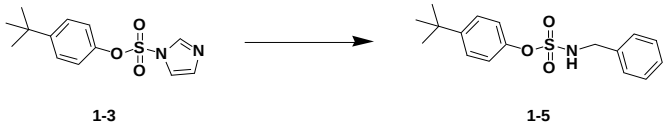
Experimental Section



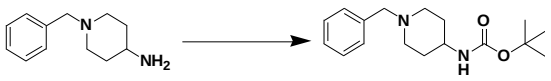
1-1 **1-2** **1-3**

Compound 1-3. A solution containing 75.0 mg (0.50 mmol) of **1-1** and 198.2 mg (1.0 mmol) of **1-2** was stirring in 8 mL of THF and then was treated with 82.5 mg (0.25 mmol) of cesium carbonate. The reaction was allowed to continue for 12 hours and then concentrated. The residue was then partitioned between the dichloromethane and water (10 mL each). The organic layer was washed with saturate ammonia chloride solution, brine, and distilled water. After drying over the anhydrous sodium sulfate, the organic layer was concentrated and chromatographed with 1:3 ethyl acetate / hexane as eluent to afford 127.8 mg (91%) of **1-3** as a clear oil: R_f 0.32 (1:3 ethyl acetate / hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (t, 1 H, J = 1.5 Hz), 7.34 (t, 1 H, J = 1.0 Hz), 7.32 (t, 1 H, J = 1.0 Hz), 7.30 (t, 1 H, J = 1.5 Hz), 7.15 (dd, 1 H, J = 1.5, 1.0 Hz), 6.81 (dd, 2 H, J = 7.0, 2.5 Hz), 1.27 (s, 9 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.1, 147.0, 137.8, 131.5, 127.4, 120.9, 118.5, 34.9, 31.5; ESI-MS m/z 281.1MH^+.



1-3 1-5

Compound 1-5. Methyl trifluoromethanesulfonate (88.4 mg, 0.54 mmol) was added dropwise into a solution of **1-3** (125.7 mg, 0.45 mmol) in 2 mL of dichloromethane at 0 ℃. After stirring at the same temperature for 3 h, the reaction was concentrated and then the residue was dissolved in 2 mL of acetonitrile. Benzyl amine (96.6 mg, 0.90 mmol) was added dropwise to the solution. The reaction mixture was allowed to stirring for an additional 12 h and then was concentrated. The residue was then partitioned between the dichloromethane and water (10 mL each). The organic layer was washed with saturate ammonia chloride solution, brine, and dried over sodium sulfate. Purification by silica gel chromatography with 1:9 ethyl acetate / hexanes as the eluent, affording 125.0 (87%) mg of **1-5** as a colorless oil: R_f 0.28 (1:9 ethyl acetate / hexane); mp; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 – 7.40 (m, 1H), 7.39 (q, 1H, J = 2.0 Hz), 7.37 (td, 1 H, J = 3.0, 2.0 Hz), 7.36 – 7.35 (m, 1 H), 7.35 – 7.33 (m, 2 H), 7.32 (dd, 1 H, J = 7.0, 3.0 Hz), 7.19 – 7.16 (m, 1H), 7.16 – 7.13 (m, 1H), 4.97 (s, 1H), 4.41 (s, 2H), 1.34 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.3, 148.0, 136.1, 129.1, 128.6, 128.4, 127.0, 121.5, 48.5, 34.8, 31.6; ESI-MS m/z 320.1MH^+.

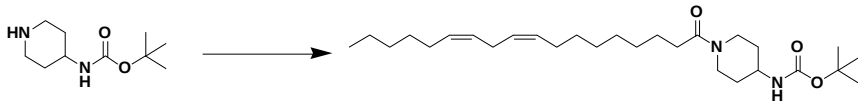


1-6 1-7

Compound 1-7. A solution containing 264 mg (6.6 mmol) of NaOH in 6.6 mL of water and 3.0 mL of *t*-BuOH was stirred at 23 ℃ for 30 min. At this point 570.9 mg (3.0 mmol) of **1-6** was added dropwise to the reaction mixture, followed by 720 mg (3.3 mmol) of di-*tert*-butyl dicarbonate. The reaction was stirred for 12 h at 23 ℃, and then was quenched with saturated aqueous sodium bicarbonate and extracted with dichloromethane (3 X 10 mL). The organic layer was dried over anhydrous sodium sulfate. The reaction was concentrated in vacuo without further purification to afford 848.9 mg (97%) of **1-7** as a white powder: mp 117-119 ℃ ; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (s, 2 H), 7.28 (s, 2 H), 7.25 – 7.19 (m, 1 H), 4.60 (s, 1 H), 3.44 (s, 3 H), 2.78 (d, 2 H, *J* = 11.0 Hz), 2.07 (t, 2 H, *J* = 11.0 Hz), 1.82 (d, 2 H, *J* = 11.5 Hz), 1.44 (s, 9 H), 1.40 (t, 2 H, *J* = 11.6 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.4, 138.7, 129.3, 128.4, 127.2, 79.3, 63.3, 52.6, 48.1, 32.9, 28.7; ESI-MS m/z 291.2 MH^+.

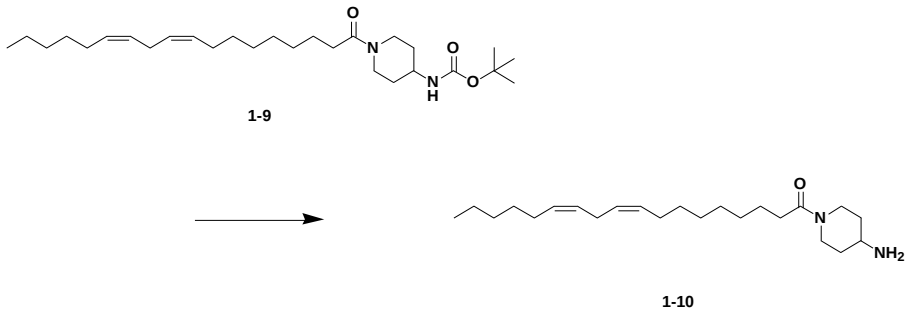
1-7 1-8

Compound 1-8. A solution containing 848.9 mg (0.82 mmol) of **1-7** in 20 mL of methanol was treated with 84.9 mg (10% by weight) of Pd/C, followed by 193 mg (0.9 mmol) of acetic acid. After stirring for overnight under a hydrogen atmosphere, the reaction mixture was filtered through a Celite pad and then concentrated. The residue was dissolved in 20 mL of dichloromethane and washed with 20 mL of saturated aqueous sodium bicarbonate. The aqueous layer was extracted with dichloromethane (2 X 15 mL). The combined organic layers were dried over anhydrous sodium sulfate and then concentrated in vacuo without further purification to afford 486.3 mg (83%) of **1-8** as a white powder: mp 157-159 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.62 (br s, 1 H), 3.52 (br s, 1 H), 3.05 (dt, 2 H, *J* = 9.5, 3.0 Hz), 2.65 (td, 2 H, *J* = 9.5, 2.5 Hz), 1.93 (d, 2 H, *J* = 11.5 Hz), 1.44 (s, 9 H), 1.42 (s, 1 H), 1.31 – 1.22 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.3, 79.3, 48.4, 45.7, 34.2, 28.6, 28.5; ESI-MS m/z 201.2 MH^+.

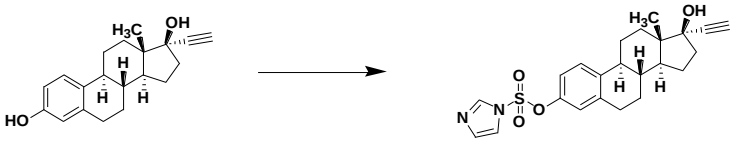


1-8 1-9

Compound 1-9. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (75.8 mg, 0.53 mmol) was added to a solution containing 89.1 mg (0.44 mmol) of **1-8**, 134.6 mg (0.48 mmol) of linoleic acid, 82.2 mg (0.53 mmol) of hydroxybenzotriazole, 53.6 mg (0.53 mmol) of triethylamine in 2 mL of acetonitrile. The mixture was stirred at 23 ℃ for 14 h, and then was quenched with saturated aqueous ammonium chloride. The reaction mixture was concentrated, and then the residue was partitioned between dichloromethane and water (20 mL each). The organic layer was washed with brine, dried over anhydrous sodium sulfate, concentrated, and then chromatographed on silica gel using 1:3 ethyl actate / hexane as eluent, affording 144.2 mg (78%) of **1-9** as a colorless oil: R_f 0.48 (1:1 ethyl acetate / hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 – 5.24 (m, 4 H), 4.65 (d, 1 H, *J* = 7.0 Hz), 4.45 (d, 1 H, *J* = 12.5 Hz), 3.75 (d, 1 H, *J* = 13.5 Hz), 3.61 (s, 1 H), 3.04 (t, 1 H, *J* = 13.5 Hz), 2.73 (t, 2 H, *J* = 7.0 Hz), 2.66 (t, 1 H, *J* = 12.5 Hz), 2.26 (t, 2 H, J = 7.0 Hz), 2.02 – 1.97 (m, 4 H), 1.96 -1.92 (m, 1 H) 1.87 (d, 1 H, *J* = 8.5 Hz), 1.63 – 1.50 (m, 2 H), 1.40 (s, 9 H), 1.35 – 1.15 (m, 16 H), 0.84 (t, 3 H, *J* =7.0 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.7, 155.3, 130.4, 130.2, 128.2, 128.1, 79.6, 48.1, 44.6, 40.7, 33.6, 33.4, 32.3, 31.7, 29.8, 29.7, 29.5, 29.3, 28.6, 27.4, 25.8, 25.6, 22.8, 14.3; ESI-MS m/z 463.4 MH^+.

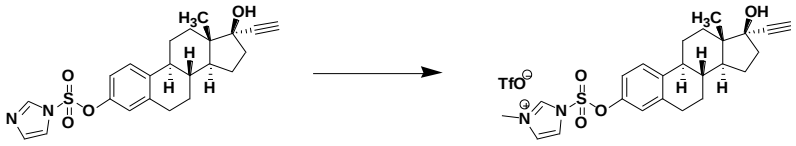


Compound 1-10. A solution of 92.5 mg (0.2 mmol) of **1-9** in 0.5 mL of dichloromethane at 0 ℃ was treated with 0.4 mL of trifluoroacetic acid, added dropwise. After stirring at the same temperature for 1 h, the reaction was diluted with 4 mL of dichloromethane, washed with 5 ml of 20% aqueous sodium hydroxide, followed by brine and then distilled water. The organic layer was dried over anhydrous sodium sulfate and then concentrated to provide 68.8 mg of crude **1-10** as a colorless oil. This material was carried to the next step without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.44 – 5.26 (m, 4 H), 4.49 (d, 1 H, *J* = 13.5 Hz), 3.80 (d, 1 H, *J* = 13.5 Hz), 3.03 (t, 1 H, *J* = 12.5 Hz), 2.88 (m, 1 H), 2.75 (t, 2 H, *J* = 6.5 Hz), 2.66 (t, 1 H, *J* = 12.5 Hz), 2.30 (t, 2 H *J* = 7.5 Hz), 2.10 – 1.96 (m, 4 H), 1.83 (t, 2 H, J = 16.0 Hz), 1.60 – 1.55 (m, 2 H), 1.39 – 1.13 (m, 18 H), 0.87 (t, 3 H, J = 5.5 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.9, 130.5, 130.3, 128.3, 128.1, 48.9, 44.6, 40.7, 33.7, 31.8, 29.9, 29.7, 29.6, 29.6, 29.4, 27.5, 27.4, 25.9, 25.7, 22.8, 14.3; ESI-MS m/z 363.3 MH^+.



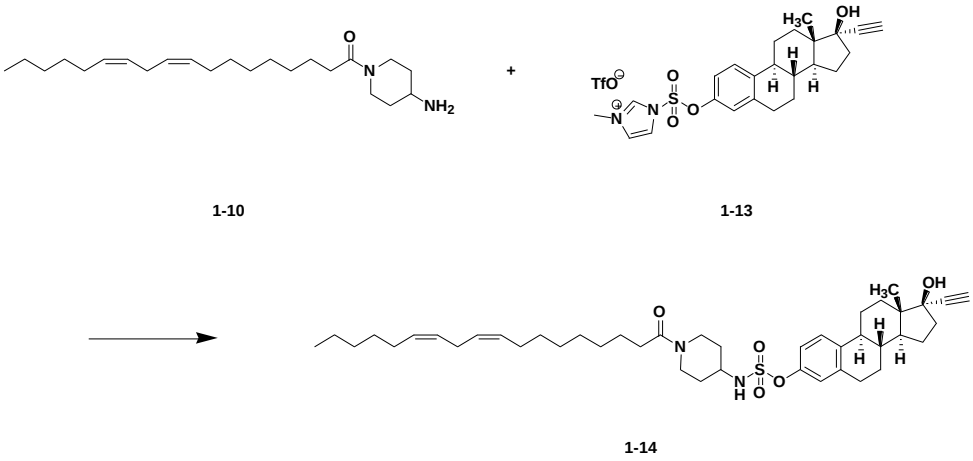
1-11 1-12

Compound 1-12. 1,1'-Sulfonyldiimidazole (118.9 mg, 0.6 mmol), **1-11** (88.9 mg, 0.3 mmol), and cesium carbonate (48.8 mg, 0.15 mmol) were dissolved in 5 mL of tetrahydrofuran. The reaction was stirred at 23 ℃ for 18 h, and then was concentrated, dissolved in 5 mL of ethyl acetate, washed with saturated aqueous ammonia chloride, and then brine, dried over anhydrous sodium sulfate, concentrated and chromatographed on silica gel using 2:3 ethyl acetate / hexane as eluent, affording 116.4 mg (91%) of **6** as a white solid: R_f 0.23 (1:3 ethyl acetate / hexane); mp 168–170 ℃ ; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (s, 1 H), 7.32 (d, 1 H, *J* = 1.5 Hz), 7.24 (d, 1 H, *J* = 9.5 Hz), 7.17 (s, 1 H), 6.63 (d, 1 H, *J* = 6.5 Hz), 6.60 (d, 1 H, *J* = 1.5 Hz), 2.80 (d, 2 H, *J* = 4.5 Hz), 2.59 (s, 2 H), 2.39 – 2.28 (m, 2 H), 2.24 – 2.18 (m, 1 H), 2.08 – 1.97 (m, 1 H), 1.97 – 1.84 (m, 2 H), 1.84 – 1.59 (m, 3 H), 1.54 – 1.22 (m, 4 H), 0.88 (s, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.1, 141.2, 139.7, 137.8, 131.4, 127.4, 121.3, 118.6, 118.2, 87.6, 79.9, 74.3, 49.7, 47.2, 43.9, 39.1, 38.9, 32.9, 29.7, 26.9, 26.3, 23.0, 12.9; ESI-MS m/z 427.1 MH^+.

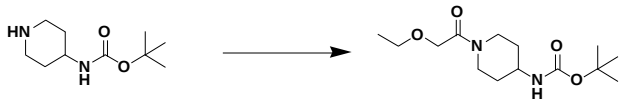


1-12 1-13

Compound 1-13. Methyl trifluoromethanesulfonate (49.2 mg, 0.3 mmol) was added dropwise to a solution of **1-12** (83.3 mg 0.2 mmol) in 0.5 mL of dichloromethane at 0 ℃. After stirring at the same temperature for an additional 3 h, the reaction mixture was concentrated to afford 113.2 mg of crude **1-13** as a hydroscopic tan solid that was used in the following step immediately without further purification.



Compound 1-14. A solution of 68.8 mg of crude **1-10** in 0.5 mL of acetonitrile was added dropwise to a solution of 113.2 mg of crude **1-13** in 0.5 mL of acetonitrile. After stirring at 23 ℃ overnight, the reaction was concentrated, and then chromatographed on silica gel by using 1:3 ethyl acetate / hexanes as the eluent to afford 74.9 mg (52% for 2 steps) of **1-14** as a colorless oil: R_f 0.44 (1:1 ethyl acetate / hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, 1 H, *J* = 9.0 Hz), 7.02 (d, 1 H, *J* = 9.0 Hz), 6.98 (s, 1 H), 5.49 (d, 1 H, *J* = 6.3 Hz), 5.40 – 5.33 (m, 4 H), 4.43 (d, 1 H, *J* = 13.5 Hz), 3.82 (d, 1 H, *J* = 13.5 Hz), 3.65 (m, 1 H), 3.10 (t, 1 H, *J* = 12 Hz), 2.85 – 2.76 (m, 4 H), 2.60 (s, 1 H), 2.43 – 2.26 (m, 5 H), 2.21 (m, 1 H), 2.19 – 2.08 (m, 1 H), 2.09 – 1.96 (m, 6 H), 1.91 – 1.89 (m, 3 H), 1.80 – 1.73 (m, 3 H), 1.57 (br s, 2 H), 1.54 – 1.40 (m, 4 H), 1.41 – 1.21 (m, 16 H), 0.89 (t, *J* = 6.5 Hz, 3 H), 0.76 (s, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 148.1, 139.4, 139.0, 130.5, 130.2, 128.3, 128.1, 126.9, 121.8, 118.9, 87.7, 80.0, 74.3, 52.4, 49.7, 47.3, 44.3, 43.9, 40.4, 39.2, 39.2, 33.6, 33.4, 32.9, 32.4, 31.7, 30.0, 29.9, 29.8, 29.7, 29.6, 29.6, 29.4, 27.4, 27.2, 26.5, 25.9, 25.6, 23.0, 22.8, 14.3, 12.9; HR-ESI MS 721.4610 (MH^+), calcd 721.4615 for $C_{43}H_{65}N_2O_5S$.

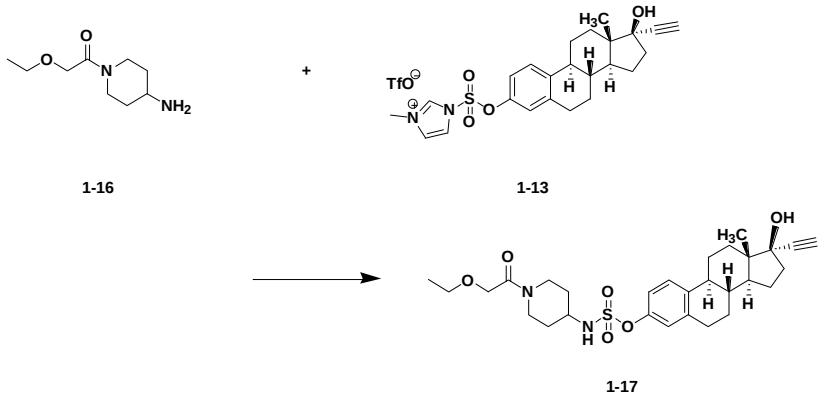


1-8 1-15

Compound 1-15. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (85.8 mg, 0.6 mmol) was added to a solution containing 100.1 mg (0.5 mmol) of **1-8**, 52.0 mg (0.5 mmol) of ethoxyacetic acid, 93.1 mg (0.6 mmol) of hydroxybenzotriazole, 60.7 mg (0.6 mmol) of triethylamine in 2.5 mL of acetonitrile. The mixture was stirred at 23 ℃ for 14 h, and then was quenched with saturated aqueous ammonium chloride. The reaction mixture was concentrated, and then the residue was partitioned between dichloromethane and water (20 mL each). The organic layer was washed with brine, dried over anhydrous sodium sulfate, concentrated, and then chromatographed on silica gel using 1:19 methanol / dichloromethane as eluent, affording 121.5 mg (85%) of **1-15** as a colorless oil: R_f 0.38 (1:15 methanol / dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.53 (s, 1 H), 4.45 (d, 1 H, *J* = 12.6 Hz), 4.13 (q, 2 H, *J* = 13.2 Hz), 3.91 (d, 1 H, *J* = 13.6 Hz), 3.68 (s, 1 H), 3.55 (m, 2 H), 3.11 (t, 1 H, *J* = 12.5 Hz), 2.77 (t, 1 H, *J* = 12.0 Hz), 1.99 (dd, 2 H, *J* = 29.0, 12.0 Hz), 1.45 (s, 9 H), 1.37 – 1.27 (m, 2 H), 1.27 (t, 3 H, *J* = 13.2 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0, 155.3, 79.8, 70.6, 67.0, 48.1, 44.2, 41.1, 33.3, 32.4, 28.6, 15.3; ESI-MS m/z 287.2 MH^+.

1-15 1-16

Compound 1-16. A solution of 121.5 mg (0.425 mmol) of **1-15** in 1.6 mL of dichloromethane at 0 ℃ was treated with 1.4 mL of trifluoroacetic acid, added dropwise. After stirring at the same temperature for 1.5 h, the reaction was diluted with 14 mL of dichloromethane, washed with 17 ml of 20% aqueous sodium hydroxide, followed by brine and then distilled water. The organic layer was dried over anhydrous sodium sulfate and then concentrated to provide 76.9 mg (97%) of crude **1-16** as a colorless oil. This material was carried to the next step without further purification. ^{1}H NMR (500 MHz,$CDCl_3$) δ 4.45 (d, 1 H, *J* = 13.0 Hz), 4.14 (q, 2 H, *J* = 13.2 Hz), 3.92 (d, 1 H, *J* = 13.5 Hz), 3.56 (m, 2H), 3.04 (t, 1 H, *J* = 13.0 Hz), 2.92 (m, 1H), 2.75 (t, 1 H, *J* = 13.0 Hz), 1.87 (d, 2 H, *J* = 29.0 Hz), 1.37 – 1.27 (m, 2 H), 1.24 (t, 3 H, *J* = 13.2 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0, 70.6, 67.0, 48.8, 44.0, 41.0, 36.3, 35.6, 15.3; ESI-MS m/z 187.1 MH^+.

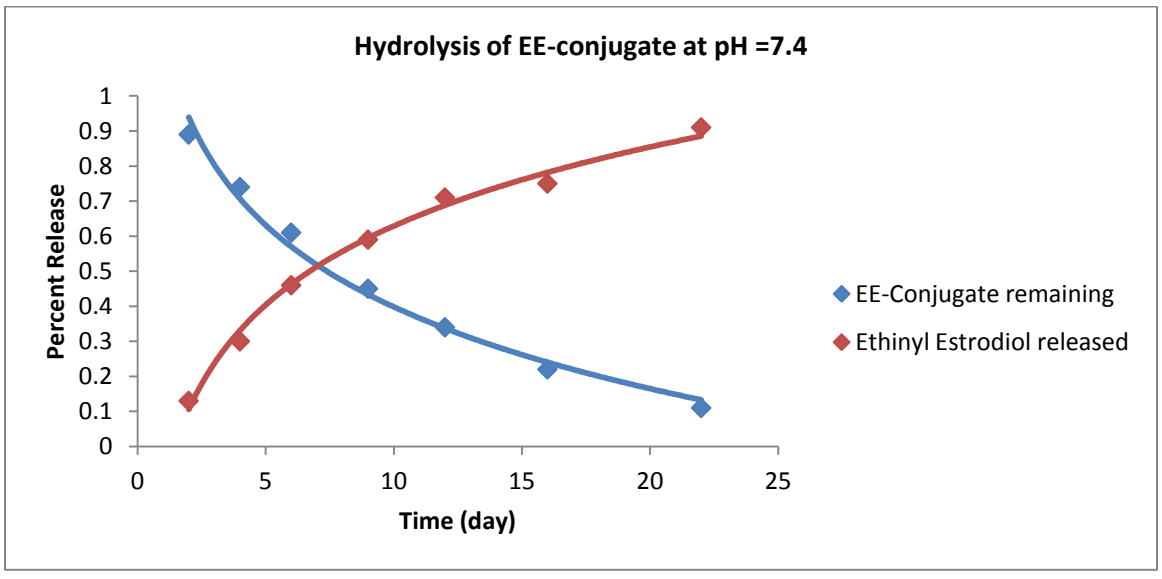


1-16 1-13 1-17

Compound 1-17. A solution of 76.9 mg of crude **1-16** in 1 mL of acetonitrile was added dropwise to a solution of 73.8 mg of crude **1-13** in 0.5 mL of acetonitrile. After stirring at 23 ℃ overnight, the reaction was concentrated, and then chromatographed on silica gel by using 1:19 methanol / dichloromethane as the eluent to afford 33.7 mg (49% for 2 steps) of **1-17** as a colorless oil: R_f 0.37 (1:15 methanol / dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, 1 H, *J* = 8.5 Hz), 7.02 (dd, 1 H, *J* = 8.5, 2.0 Hz), 6.98 (s, 1 H), 5.29 (s, 1 H), 5.08 (d, 1 H, *J* = 7.2 Hz), 4.40 (d, 1 H, *J* = 12.7 Hz), 4.11 (q, 2 H *J* = 13.2 Hz), 3.93 (d, 1 H, *J* = 12.7 Hz), 3.67 (m, 1 H), 3.60 – 3.46 (m, 2 H), 3.14 (t, 1 H, *J* = 11.9 Hz), 2.86 (t, 3 H, *J* = 11.0 Hz), 2.60 (s, 1 H), 2.39-2.24 (m, 2H), 2.23 (t, 1 H, *J* = 10.5 Hz), 2.19 – 2.10 (m, 1 H), 2.09 – 2.05 (m, 2 H), 2.01 – 1.85 (m, 2 H), 1.84 – 1.60 (m, 4 H), 1.58 – 1.29 (m, 5 H), 1.22 (t, *J* = 7.0 Hz, 3H), 0.87 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2, 148.1, 139.4, 139.0, 127.0, 121.8, 118.9, 87.6, 80.0, 74.4, 70.6, 67.1, 52.3, 49.7, 47.3, 43.9, 43.8, 40.8, 39.2, 39.2, 33.4, 32.9, 32.5, 29.8, 27.2, 26.5, 23.0, 15.3, 12.9; ESI-MS *m/z* 545 MH^+.

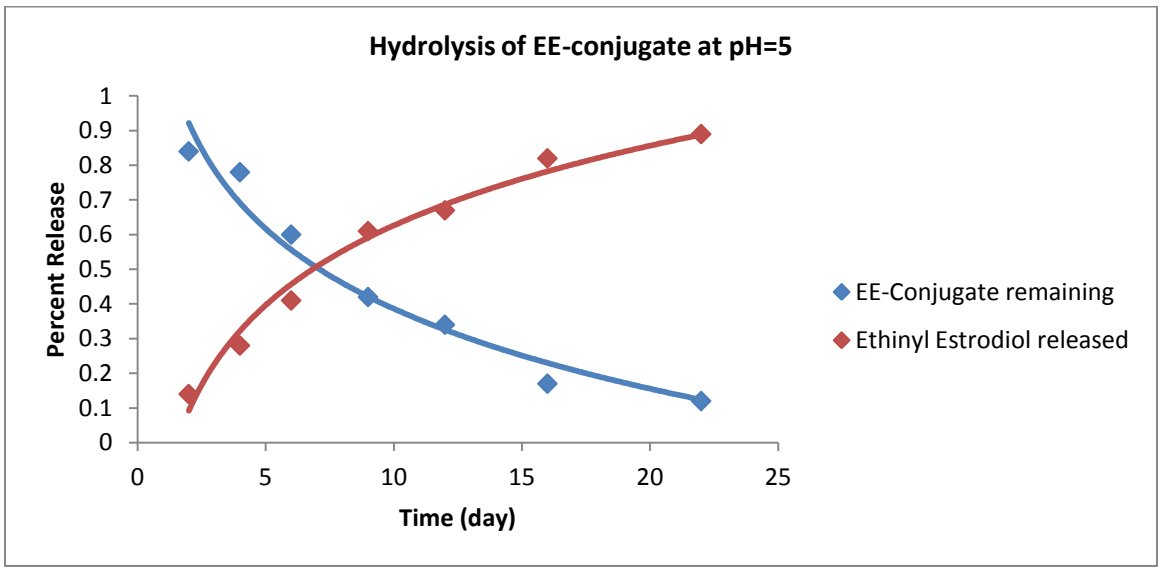
Hydrolysis Study on **1-14** at pH=7.4

Estradiol conjugate **1-14** (20.6 mg 0.0286 mmol) was dissolved in 30 mL of a 9:4 ethanol/0.05M aqueous phosphate buffer (pH=7.4) mixture, and the solution was shaken in a 37 ℃ incubator for 22 days. Seven 4 mL aliquots were collected at 2-, 4-, 6-, 9-, 12-, 16-, and 22-day intervals. Estradiol release was quantified by adding 38.0 µL of a 0.1 M solution of 1,4-dinitrobezene in deuterochloroform as an internal standard, then comparing integrals of ^{1}H NMR signals corresponding to those of the conjugate steroid (dd at 7.03 ppm, 1 H, and d at 7.00 ppm, 1 H), ethinyl estradiol (dd at 6.63 ppm, 1H, d at 6.56 ppm, 1 H), and the internal standard (aromatic protons at 8.43 ppm, s, 4H).



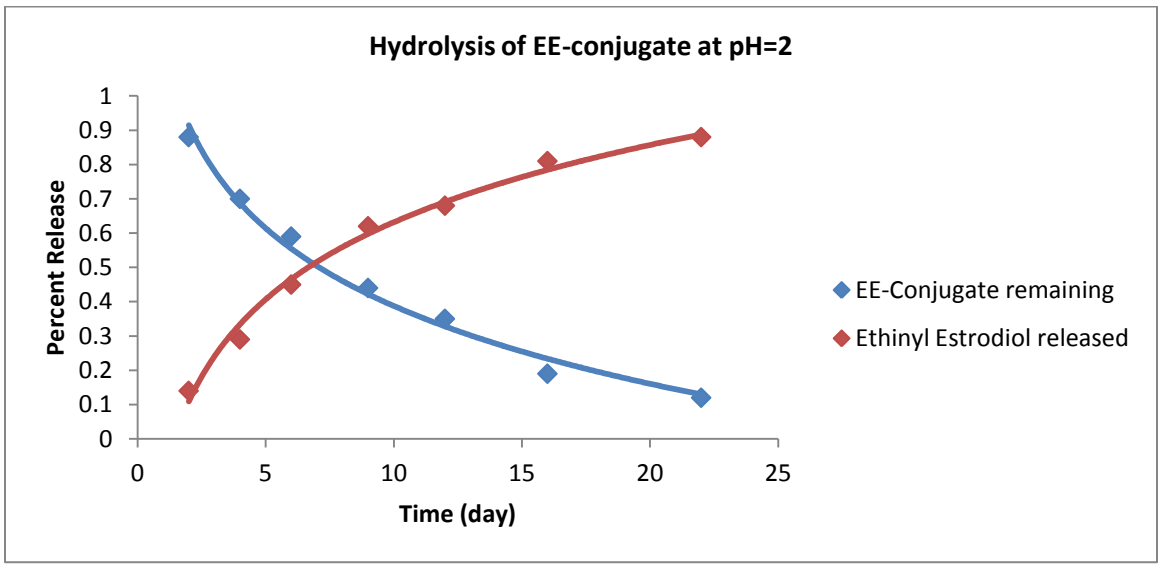
Hydrolysis Study on **1-14** at pH=5

Estradiol conjugate **1-14** (20.3 mg 0.0282 mmol) was dissolved in 30 mL of a 9:4 ethanol/0.05M aqueous acetate buffer (pH=5) mixture, and the solution was shaken in a 37 ℃ incubator for 22 days. Seven 4 mL aliquots were collected at 2-, 4-, 6-, 9-, 12-, 16-, and 22-day intervals. Estradiol release was quantified by adding 37.5 µL of a 0.1 M solution of 1,4-dinitrobezene in deuterochloroform as an internal standard, then comparing integrals of ^{1}H NMR signals corresponding to those of the conjugate steroid (dd at 7.03 ppm, 1 H, and d at 7.00 ppm, 1 H), ethinyl estradiol (dd at 6.63 ppm, 1H, d at 6.56 ppm, 1 H), and the internal standard (aromatic protons at 8.43 ppm, s, 4H).



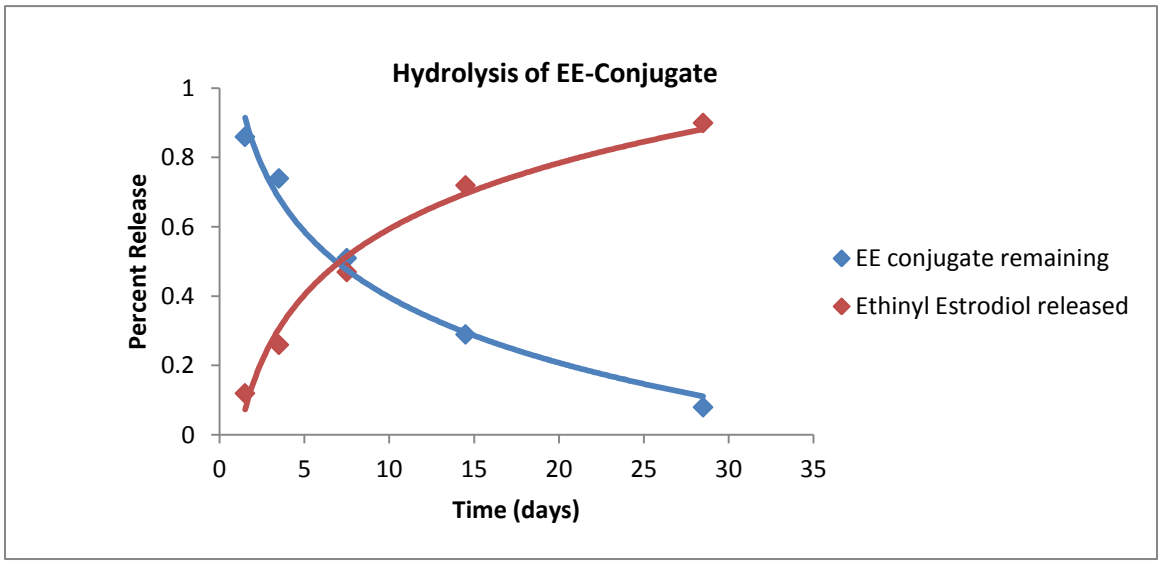
Hydrolysis Study on **1-14** at pH=2

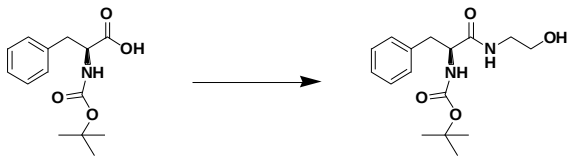
Estradiol conjugate **1-14** (19.5 mg 0.0270 mmol) was dissolved in 30 mL of a 9:4 ethanol/0.05M aqueous hydrogen chloride buffer (pH=2) mixture, and the solution was shaken in a 37 ℃ incubator for 22 days. Seven 4 mL aliquots were collected at 2-, 4-, 6-, 9-, 12-, 16-, and 22-day intervals. Estradiol release was quantified by adding 36.0 μL of a 0.1 M solution of 1,4-dinitrobezene in deuterochloroform as an internal standard, then comparing integrals of ^{1}H NMR signals corresponding to those of the conjugate steroid (dd at 7.03 ppm, 1 H, and d at 7.00 ppm, 1 H), ethinyl estradiol (dd at 6.63 ppm, 1H, d at 6.56 ppm, 1 H), and the internal standard (aromatic protons at 8.43 ppm, s, 4H).



Hydrolysis Study on **1-17** at pH=7.4

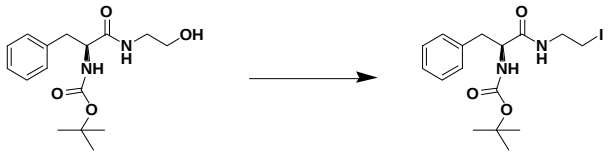
Estradiol conjugate **1-17** (20.0 mg 0.0367 mmol) was dissolved in 30 mL of a 9:4 ethanol/0.05M aqueous phosphate buffer (pH=7.4) mixture, and the solution was shaken in a 37 ℃ incubator for 28.5 days. Six 4 mL aliquots were collected at 0.5-, 1.5-, 3.5-, 7.5-, 14.5-, and 28.5- intervals. Estradiol release was quantified by adding 48.9 µL of a 0.1 M solution of 1,4-dinitrobezene in deuterochloroform as an internal standard, then comparing integrals of ^{1}H NMR signals corresponding to those of the conjugate steroid (dd at 7.02 ppm, 1 H, and d at 6.98 ppm, 1 H), ethinyl estradiol (dd at 6.63 ppm, 1H, d at 6.56 ppm, 1 H), and the internal standard (aromatic protons at 8.43 ppm, s, 4H).





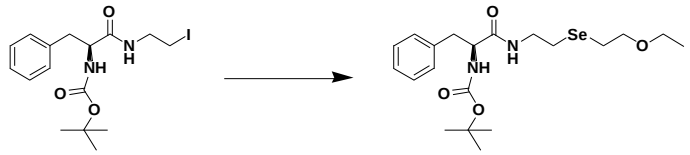
2-1 2-2

Compound 2-2. A stirring solution of 265.3 mg (1.0 mmol) of N-Boc phenylalanine in 8 mL of acetonitrile was treated with 183.6 mg of hydroxylbenzotriazole (1.2 mmol), followed by dropwise addition of 387.5 mg (3.0 mmol) of diisopropylethylamine and 79.4 mg (1.3 mmol) of 2-aminoethanol. After stirring for 15 min at 0 ℃, 230.4 mg (1.2 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide was added and the reaction mixture was stirred for an additional 12 h at room temperature. The reaction was quenched with aqueous saturate sodium bicarbonate (10 mL) and then dissolved in dichloromethane. The aqueous layer was extracted with dichloromethane (3×10 mL). The organic layers were combined, washed with brine, and then dried over sodium sulfate. After the concentration of the solution, the crude product was purified by chromatography with 3% methanol in dichloromethane as the eluent to produce 240.5 mg (78%) of **2-2** as a colorless oil: R_f 0.26 (1:15 methanol / dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 – 7.22 (m, 2 H), 7.19 (t, 2 H, J = 8.0 Hz), 7.06 (s, 1 H), 5.62 (d, 1 H, J = 7.0 Hz), 4.39 (bs, 1H), 3.55 (bs, 2 H), 3.50 (bs, 1 H), 3.23 (m, 2 H), 3.11 – 2.90 (m, 2 H), 1.35 (s, 9 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.7, 156.0, 137.1, 129.6, 128.7, 127.0, 80.3, 61.5, 56.3, 42.4, 39.2, 28.5; ESI-MS m/z 309.2 MH^+.



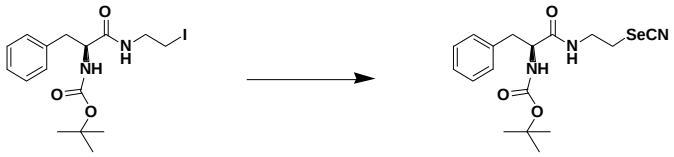
2-2 2-3

Compound 2-3. A solution of 240.0 mg (0.785 mmol) of **2-2** in 6 mL of THF was treated with 411.5 mg (1.57 mmol) of triphenylphosphine and 106.8 mg (1.57 mmol) of imidazole at room temperature. After stirring for 5 min, 398.4 mg (1.57 mmol) of iodine was added to the solution and the reaction mixture was allowed to stir for an additional 1 h at the same temperature. The reaction mixture was concentrated, dissolved in 20 mL of dichloromethane, and then the solution was washed with 10 mL of 5% $Na_2S_2O_3$ solution, and 10 mL of brine. After being dried over sodium sulfate, the solvent was evaporated and the residue was purified by chromatography with 3:7 ethyl acetate / hexanes as the eluent to produce 248.3 mg (76%) of **2-3** as a yellow solid: mp 130–132 ℃; R_f 0.43 (3:7 ethyl acetate / hexanes); ^{1}H NMR (500 MHz, cdcl$_3$) δ 7.37 – 7.26 (m, 2 H), 7.24 (d, 1 H, J = 7.0 Hz), 7.21 – 7.12 (m, 2 H), 6.55 (s, 1 H), 5.23 (d, 1 H, J = 7.0 Hz), 4.36 (bs, 1 H), 3.55 (m, 1 H), 3.47 (m, 1 H), 3.12 (m, 1 H), 3.05 (m, 3 H), 1.40 (s, 9 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.6, 155.7, 136.9, 129.5, 128.9, 127.2, 80.4, 56.1, 42.0, 38.9, 28.6, 4.3; ESI-MS m/z 419.1 MH^+.



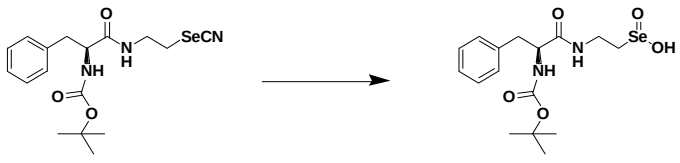
2-3 2-4

Compound 2-4. A solution of 52.4 mg of **2-3** in 0.4 mL of THF was added into a solution of sodium ethoxyethyl selenonate [prepared by treating a solution of 57.2 mg (0.188 mmol) of bisethoxyethyl diselenide in ethanol with 15.6 mg (0.413 mmol) of sodium borohydride in ethanol] at room temperature. After stirring for 30 min at the same temperature, 7 mL of water was added into the reaction mixture, and the pH of the solution was adjusted to pH 7-8 by acetic acid. The reaction mixture was extracted by ethyl acetate (10 mL×3). The organic layers were combined, washed with brine, and dried over sodium sulfate. The reaction mixture was concentrated and then chromatographed by 3:7 ethyl acetate / hexane as eluent to give 39.1 mg (71%) of **2-4** as a colorless oil: R_f 0.16 (3:7 ethyl acetate / hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.26 (m, 2 H), 7.23 (d, 1 H, *J* = 7.0 Hz), 7.20 (m, 2 H), 6.37 (bs, 1H), 5.15 (bs, 1H), 4.31 (bs, 1H), 3.62 (t, 2 H, *J* = 6.5 Hz), 3.50 (q, 2 H, J = 7.0 Hz), 3.49 – 3.45 (m, 1 H), 3.40 (m , 1H), 3.04 (bs, 2 H), 2.65 (t, 2 H, *J* = 6.5 Hz), 2.69 – 2.56 (m, 1H), 2.52 (bs, 1H), 1.41 (s, 9H), 1.20 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.2, 155.8, 137.0, 129.5, 128.8, 127.1, 81.4, 70.9, 66.5, 56.2, 39.6, 39.1, 28.5, 23.8, 23.2, 15.4; ESI-MS m/z 467.2 MNa^+.



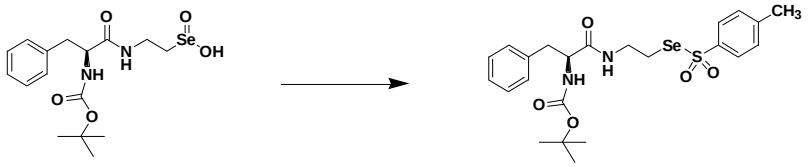
2-3 2-5

Compound 2-5. A solution of 232.1 mg (0.555 mmol) of **2-3** in 3 mL of acetone was treated with 120.0 mg (0.832 mmol) of KSeCN. After stirring for 3 h at room temperature, the reaction mixture was concentrated. And then the residue was partitioned between dichloromethane and aqueous ammonia chloride. The organic layer was combined and dried over sodium sulfate. 203.7 mg (93%) of product **2-5** was obtained as a white solid by chromatographic purification with 3% methanol in dichloromethane as the eluent: mp 146–148 ℃; R_f 0.32 (3% methanol in dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 (m, 2 H), 7.28 – 7.23 (m, 1 H), 7.17 (m, 2 H), 6.55 (bs, 1H), 5.10 (d, 1 H, J = 7.0 Hz), 4.32 (m, 1 H), 3.67 (m, 1 H), 3.57 (bs, 1H), 3.12 (m, 1 H), 3.06 (d, 2 H, J = 6.5 Hz), 2.98 (m, 1 H), 1.41 (s, 9 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.3, 155.7, 136.8, 129.5, 129.0, 127.4, 101.6, 80.7, 56.1, 40.1, 38.6, 29.1, 28.5; ^{77}Se NMR (95 MHz, $CDCl_3$) δ 199.7 (vs PhSeSePh at 460.0 ppm as an external standard); ESI-MS m/z 420.0 MNa^+.



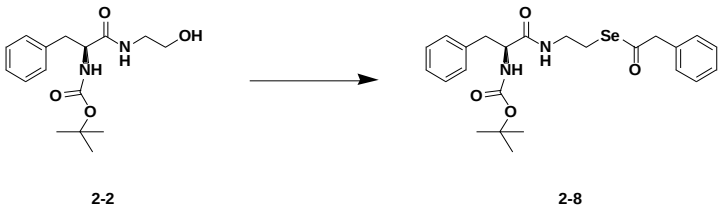
2-5 2-6

Compound 2-6. Dimethyldioxirane was added to a stirred solution of 110.8 mg (0.28 mmol) of **2-5** in 7 mL of dichloromethane until all of **2-5** was consumed according to TLC analysis (total 1.05 mL of 0.4 M solution of DMDO in chloroform). The reaction mixture was concentrated and then chromatographed on silica gel with 8% methanol in dichloromethane as the eluent to give 77.3 mg (70%) of **2-6** as a colorless oil: R_f 0.26 (2:23 methanol / dichloromethane); ^{1}H NMR (500 MHz, CD_3OD) δ 7.39 – 7.27 (m, 2 H), 7.24 (m, 3 H), 6.74 (s, 1 H), 4.35 – 4.13 (m, 1 H), 3.74 – 3.57 (m, 1 H), 3.51 (m, 1 H), 3.31 (dt, J = 1.5 Hz, 1 H), 3.15 – 3.02 (m, 2 H), 3.01 – 2.90 (m, 1 H), 2.91 – 2.74 (m, 2 H), 1.38 (s, 9 H); ^{13}C NMR (126 MHz, CD_3OD) δ 174.2, 156.4, 137.4, 129.3, 128.3, 126.7, 79.6, 57.2, 56.2, 38.0, 33.3, 27.5; ^{77}Se NMR (95 MHz, CD_3OD) δ 1294.1, 1292.9 (vs PhSeSePh at 460.0 ppm as an external standard); ESI-MS m/z 440.4 as the seleninate methyl ester ·Na^+.



2-6 2-7

Compound 2-7. A solution of seleninic acid **2-6** (22.5 mg, 0.056 mmol) in 3 mL of dichoromethane was added dropwise by syringe to a solution of 11.3 mg (0.061 mmol) of p-toluenesulfonyl hydrazide in dichloromethane. After 30 min of stirring at room temperature, the reaction mixture was concentrated and chromatographed on silica with 3:7 ethyl acetate / hexanes as the eluent to give 25.9 (88%) of selenosulfonate **2-7** as a yellow oil: R_f 0.23 (1:1 ethyl acetate / hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, 2 H, J = 7.5 Hz), 7.35 – 7.33 (m, 2 H), 7.30 – 7.25 (m, 2 H), 7.23 (d, 1 H, 6.5 Hz), 7.20 – 7.17 (m, 2 H), 6.44 (bs, 1 H), 5.08 (bs, 1 H), 4.32 (bs 1 H), 3.60 (m, 2 H), 3.22 (m, 1 H), 3.15 (bs, 1 H), 3.04 (d, 2 H, J = 4.5 Hz), 2.45 (s, 3 H), 1.40 (s, 9 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.9, 155.8, 145.2, 144.1, 136.9, 130.1, 129.5, 128.9, 127.3, 126.8, 82.3, 56.2, 39.4, 38.8, 33.0, 28.5, 21.9; ^{77}Se NMR (95 MHz, $CDCl_3$) δ 856.9 (vs PhSeSePh at 460.0 ppm as an external standard); ESI-MS m/z 549.1 MNa^+.

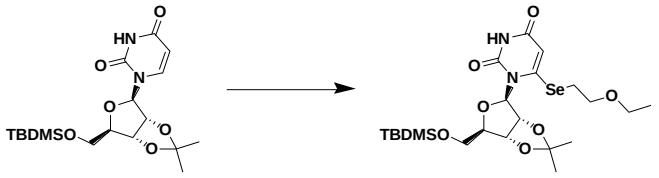


2-2 2-8

Compound 2-8. A solution of 125.8 mg (0.48 mmol) of triphenylphosphine in 1.5 mL of tetrahedrofuran was stirred at -20 ℃. Diisopropyl azodicarboxylate (97.1 mg, 0.48 mmol) was added dropwise and the reaction mixture was maintained at -20 ℃ until the white phosphonium intermediate formed. The reaction mixture was then cooled to -50 ℃, and a solution of 74.0 mg (0.24 mmol) of **2-2** was added dropwise by syringe. After 5 min of stirring, 2 mL of a toluene solution of (2-phenyl)-selenoacetic acid [prepared by heating at reflux a 2 mL toluene solution of 100 mg (0.734 mmol) of phenyl acetic acid and 117 mg of Woollins's reagent for 2h] was added by cannula and the reaction mixture was allowed to warm to 23 ℃, and then was stirred for an additional 2h. The solution was concentrated and chromatographed on silica with 3:7 ethyl acetate / hexanes as eluent to afford 77 mg (66%) of **2-8** as a colorless oil: R_f 0.27 (3:7 ethyl acetate / hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.30 (m, 4 H), 7.30 – 7.25 (m, 4 H), 7.17 (d, 2 H, J = 7.0 Hz), 6.15 (bs, 1 H), 5.03 (bs, 1 H), 4.28 (bs, 1 H), 3.83 (s, 2 H), 3.44 (m, 1 H), 3.33 (bs, 1 H), 3.01 (d, 2 H, J = 6.0 Hz), 2.88 (m, 1 H), 2.82 (m, 1 H), 1.41 (s, 9 H); ^{13}C NMR (126 MHz, CD_3OD) δ 200.7, 171.4, 155.8, 136.9, 132.9, 130.2, 129.5, 129.0, 128.9, 128.0, 127.1, 80.4, 56.2, 54.3, 40.1, 39.0, 28.5, 25.1; ^{77}Se NMR (95 MHz, $CDCl_3$) δ 547.6 (vs PhSeSePh at 460.0 ppm as an external standard); ESI-MS m/z 513.1 MNa^+.

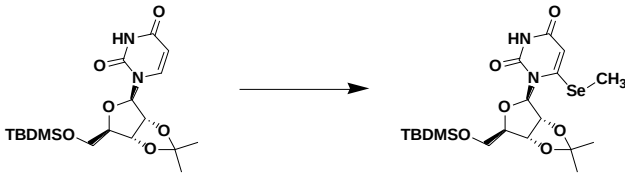
2-8 2-6

Compound 2-6. Dimethyldioxirane was added to a stirred solution of 43.4 mg (0.089 mmol) of **2-5** in 1 mL of dichloromethane until all of **2-8** was consumed according to TLC analysis (total 0.488 mL of 0.4 M solution of DMDO in chloroform). The reaction mixture was concentrated and then chromatographed on silica with 8% methanol in dichloromethane as the eluent to give 22.6 mg (63%) of **2-6** as a colorless oil. The R_f and NMR spectrum matched those of authentic **2-6** as prepared previously.



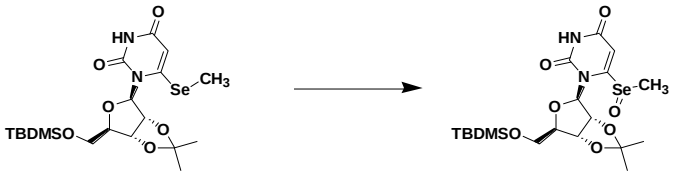
3-2 3-3

Compound 3-3. A stirring solution of LDA (1.8 mL, 0.9 mmol in THF) in anhydrous THF was treated with a solution of 119.5 mg (0.30 mmol) of compound **3-2** in 4 mL of anhydrous THF at -78 ℃. After stirring for 1 h. A solution of bis(ethoxyethyl) diselenide (136.9 mg, 0.45 mmol) in anhydrous THF (1 mL) was added and the mixture was stirred for an additional 5 h at the same temperature. The reaction was quenched with ammonia chloride solution, brought to room temperature, and then dissolved in ethyl acetate (20 mL). The organic layer was washed with saturated $NaHCO_3$ solution (10 mL), brine (10 mL), and then dried over sodium sulfate. Evaporation of the solvent and purification of the crude by column chromatography (5% acetone in dichloromethane as eluent) gave 80 .6 mg (48%) of **3-3** as a yellow oil: R_f 0.32 (1:9 acetone / dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (s, 1 H), 6.03 (s, 1 H), 5.79 (s, 1 H), 5.16 (d, 1 H, J = 6.5 Hz), 4.77 (t, 1 H, J = 5.0 Hz), 4.10 (dd, 1 H, J = 10.5, 5.5 Hz), 3.76 (m, 2 H), 3.73 (t, 2 H, J = 4.5 Hz), 3.48 (q, 2 H, J = 6.0 Hz), 3.14 (t, 2 H, J = 6.0 Hz), 1.50 (s, 3 H), 1.29 (s, 3 H), 1.17 (t, 3 H, J = 6.5 Hz), 0.84 (s, 9 H), 0.01 (s, 6 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.0, 153.8, 149.6, 114.2, 104.3, 95.2, 89.6, 84.5, 82.2, 68.3, 66.9, 64.1, 30.3, 26.2, 26.1, 25.6, 18.6, 15.3, -5.0, -5.0; ^{77}Se NMR (95 MHz, $CDCl_3$) δ 320.5 (vs PhSeSePh at 460.0 ppm as an external standard); ESI-MS m/z 573.2 MNa^+.



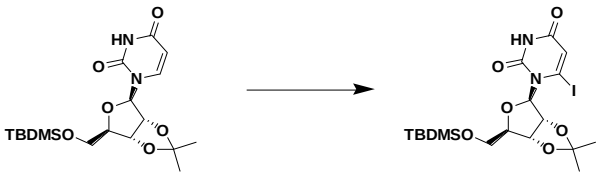
3-2 3-4

Compound 3-4. A stirring solution of LDA (1.8 mL, 0.9 mmol in THF) in anhydrous THF was treated with a solution of 115.4 mg (0.30 mmol) of compound **3-2** in 4 mL of anhydrous THF at -78 ℃. After stirring for 1 h, a solution of dimethyl diselenide (67.7 mg, 0.36 mmol) in anhydrous THF (1 mL) was added and the mixture was stirred for an additional 5 h at the same temperature. The reaction was quenched with ammonia chloride solution, brought to room temperature, and then dissolved in ethyl acetate (20 mL). The organic layer was washed with saturated $NaHCO_3$ solution (10 mL), brine (10 mL), and then dried over sodium sulfate. Evaporation of the solvent and purification of the crude by column chromatography (5% acetone in dichloromethane as eluent) gave 79.6 mg (54%) of **3-4** as a yellow oil: R_f 0.43 (1:9 acetone / dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.50 (bs, 1 H), 5.88 (d, 1 H, J = 2.0 Hz), 5.61 (s, 1 H), 5.17 (dd, 1 H, J = 6.5, 1.5 Hz), 4.79 (dd, 1 H, J = 6.5, 4.0 Hz), 4.10 (dd, 1 H, J = 10.5, 6.0 Hz), 3.77 (dd, 2 H, J = 6.0, 2.5 Hz), 2.33 (s, 3 H), 1.52 (s, 3 H), 1.29 (s, 3 H), 0.84 (s, 9 H), 0.00 (s, 6 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.6, 155.4, 149.8, 114.2, 101.8, 94.9, 89.7, 84.5, 82.1, 64.1, 27.4, 26.1, 25.6, 18.7, 10.0, -5.0, -5.0; ^{77}Se NMR (95 MHz, $CDCl_3$) δ 259.7 (vs PhSeSePh at 460.0 ppm as an external standard); ESI-MS m/z 515.2 MNa^+.



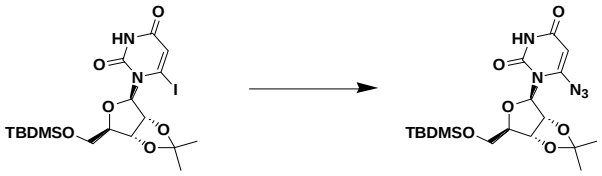
3-4 3-5

Compound 3-5. Dimethyldioxirane was added to a stirred solution of 25.4 mg (0.052 mmol) of **3-4** in 1 mL of dichloromethane until all of **3-4** was consumed according to TLC analysis (total 0.13 mL of 0.4 M solution of DMDO in chloroform). The reaction mixture was concentrated to give 24.9 mg (94%) of **3-5** as a colorless oil: ^{1}H NMR (500 MHz, d-DMSO) δ 11.61 (s, 1 H), 6.24 (s, 1H), 5.94 (d, 1 H, *J* = 3.5 Hz), 5.02 (dd, 1 H, *J* = 7.0, 3.5 Hz), 4.62 (dd, 1 H, *J* = 7.0, 5.5 Hz), 3.99 (dd, 1 H, *J* = 8.0, 5.5 Hz), 3.84 (m, 2 H), 2.52 (s, 3 H), 1.41 (s, 3 H), 1.21 (s, 3 H), 0.80 (s, 9 H), 0.00 (s, 6H); ^{13}C NMR (126 MHz, d-DMSO) δ 161.7, 161.3, 150.6, 115.2, 101.1 92.3, 86.4, 84.6, 79.7, 62.6, 27.5, 26.5, 25.8, 18.7, -4.7, -4.9; ^{77}Se NMR (95 MHz, $CDCl_3$) δ 897.6 (vs PhSeSePh at 460.0 ppm as an external standard); ESI-MS m/z 531.1 MNa^+.



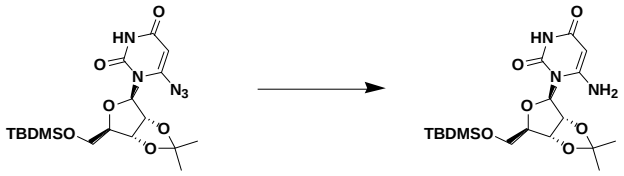
3-2 3-6

Compound 3-6. A stirring solution of LDA (9.0 mL, 4.5 mmol in THF) in anhydrous THF was treated with a solution of 594.4 mg (1.55 mmol) of compound **3-2** in 18 mL of anhydrous THF at -78 ℃. After stirring for 1 h, a solution of iodine (590.0 mg, 2.3 mmol) in anhydrous THF (4 mL) was added and the mixture was stirred for an additional 5 h at the same temperature. The reaction was quenched with saturate ammonia chloride solution, brought to room temperature and then dissolved in ethyl acetate (40 mL). The organic layer was washed with saturated $NaHCO_3$ solution (20 mL), 5% $Na_2S_2O_3$ solution (20 mL), brine (20 mL), and then dried over sodium sulfate. Evaporation of the solvent and purification of the crude by column chromatography (3:7 ethyl acetate / hexanes as eluent) gave 538 mg (66%) of **3-6** as a yellow oil: R_f 0.35 (3:7 ethyl acetate / hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.43 (bs, 1H), 6.41 (s, 1 H), 6.05 (d, 1 H, J = 1.0 Hz), 5.14 (dd, 1 H, J = 6.0, 1.0 Hz), 4.77 (dd, 1 H, J = 6.0, 4.5 Hz), 4.13 (t, 1 H, J = 5.0 Hz), 3.89 – 3.40 (dd, 2 H, J = 7.5 Hz, 1.5 Hz), 1.51 (s, 3 H), 1.27 (s, 3 H), 0.82 (s, 9 H), 0.00 (s, 6 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.3, 147.6,117.0, 114.1, 114.0, 102.1, 90.2, 84.7, 82.2, 64.3, 27.5, 26.2, 25.6, 18.7, -4.95, -4.97; ESI-MS m/z 525.1 MH^+.



3-6 3-7

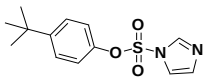
Compound 3-7. Compound **3-6** (54.2 mg, 0.103 mmol) was dissolved in 0.5 mL of anhydrous N,N-dimethylformamide, and NaN_3 (7.4 mg, 0.114 mmol) was added into the solution. The reaction mixture was stirred at room temperature for 1 h in the dark. Organic solvent was evaporated under vacuum and the crude residue was dissolved in ethyl acetate (15 mL), washed with brine, and then dried over Na_2SO_4. The combined organic layers were evaporated and the crude product was purified by silica gel column chromatography (1:1 ethyl acetate / hexanes as eluent) to give 41.3 mg of compound **3-7** (91%) as a light brown oil: R_f 0.35 (1:1 ethyl acetate / hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.05 (bs, 1 H), 6.04 (d, 1 H, *J* = 1.5 Hz), 5.47 (s, 1 H), 5.09 (dd, 1 H, *J* = 6.0, 1.5 Hz), 4.76 (dd, 1 H, *J* = 6.5, 5.0 Hz), 4.07 (dd, 1 H, *J* = 5.0, 1.5 Hz), 3.76 (dd, 2 H, J = 5.0, 2.0 Hz), 1.51 (s, 3 H), 1.23 (s, 3 H), 0.83 (s, 9 H), 0.01 (s, 6 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.7, 152.4, 149.6, 114.2, 90.0, 89.6, 88.8, 84.4, 82.0, 64.2, 27.5, 26.2, 25.6, 18.7, -5.0, -5.1; ESI-MS m/z 440.2 MH^+.



3-7 3-8

Compound 3-8. A solution containing 116.6 mg (0.27 mmol) of **3-7** in 4 mL of methanol was treated with 11.6 mg (10 % by weight) of Pd/C. After stirring for 3 h under a hydrogen atmosphere, the reaction mixture was filtered through a celite pad and then concentrated without further purification to afford 106.7 mg (97%) of **3-8** as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.39 (d, 1 H, *J* = 3.5 Hz), 5.67 (s, 1 H), 4.99 (dd, 1 H, J = 6.5, 3.5 Hz), 4.89 (s, 1 H), 4.75 (t, 1 H, *J* =6.0 Hz,), 3.96 (bs, 1 H), 3.82 (m, 2 H), 1.47 (s, 3 H), 1.25 (s, 3 H), 0.81 (s, 9 H), 0.02 (s, 6 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.8, 156.1, 150.8, 115.7, 89.1,84.6, 82.3, 80.4, 78.9, 62.3, 27.4, 26.2, 25.7, 18.8, -5.2, -5.2; ESI-MS m/z 414.2 MH^+.

Appendix



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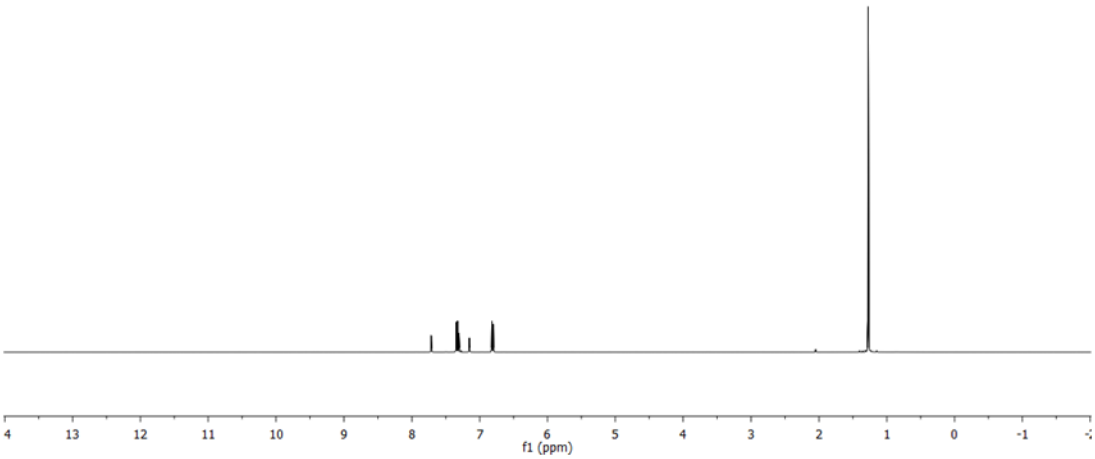
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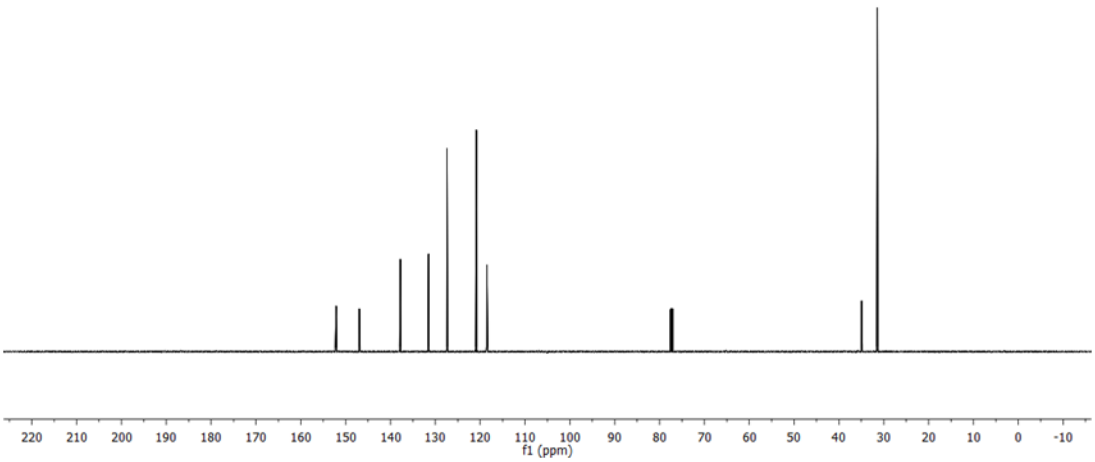
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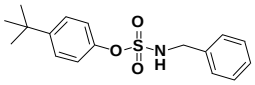

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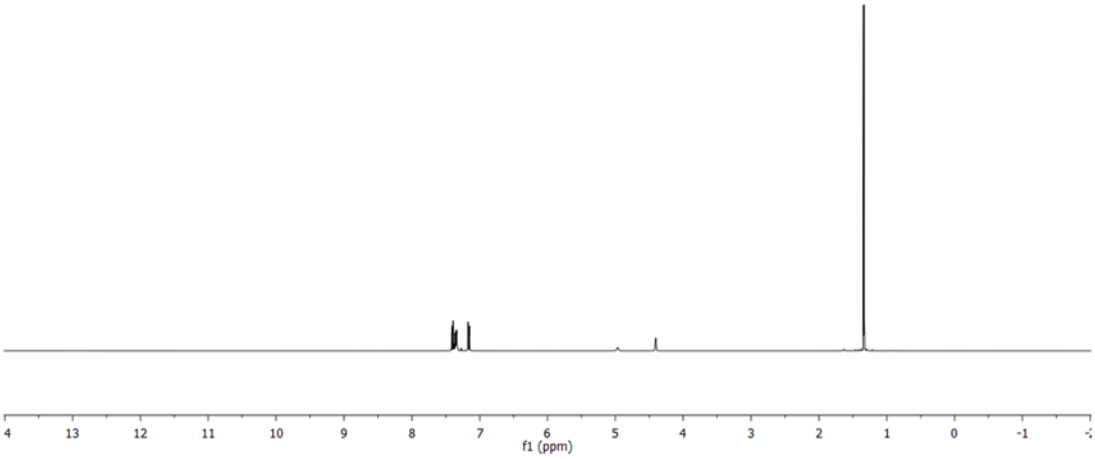
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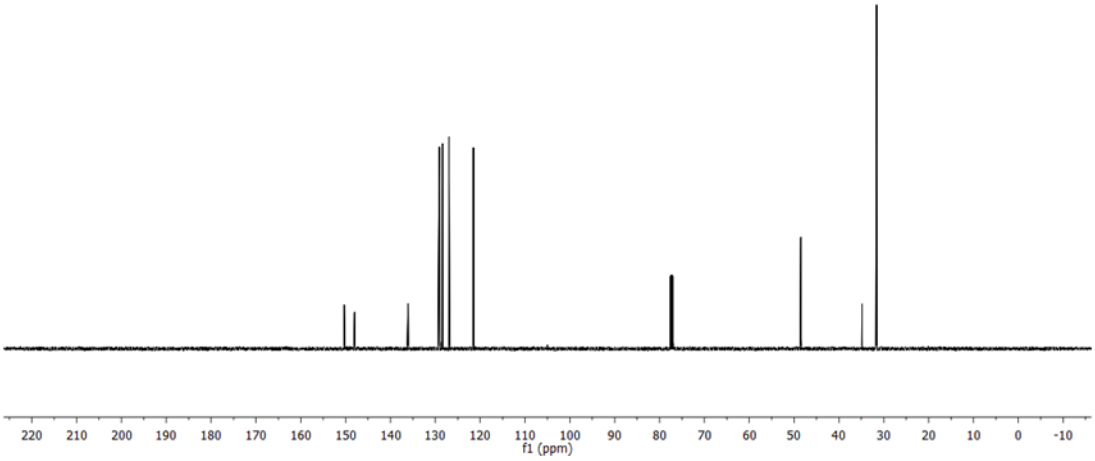
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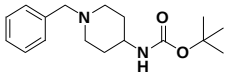

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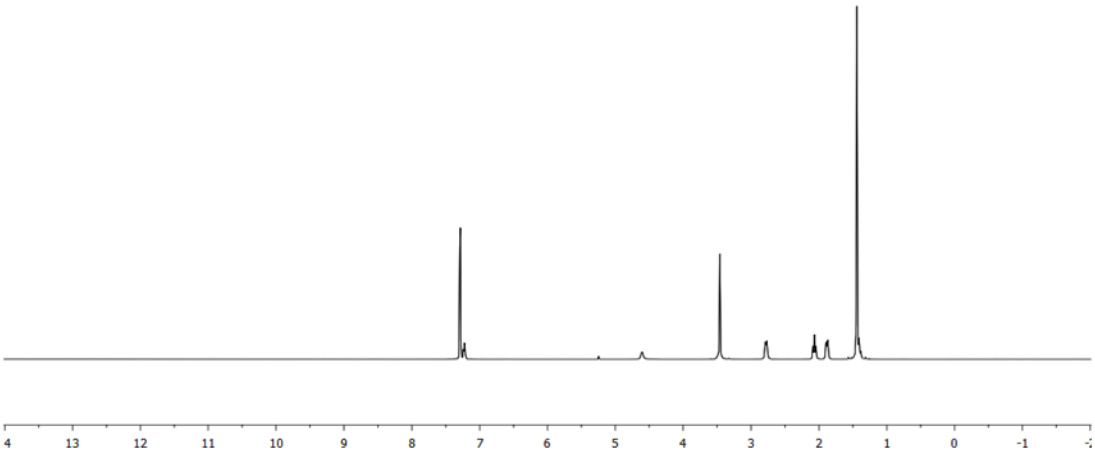
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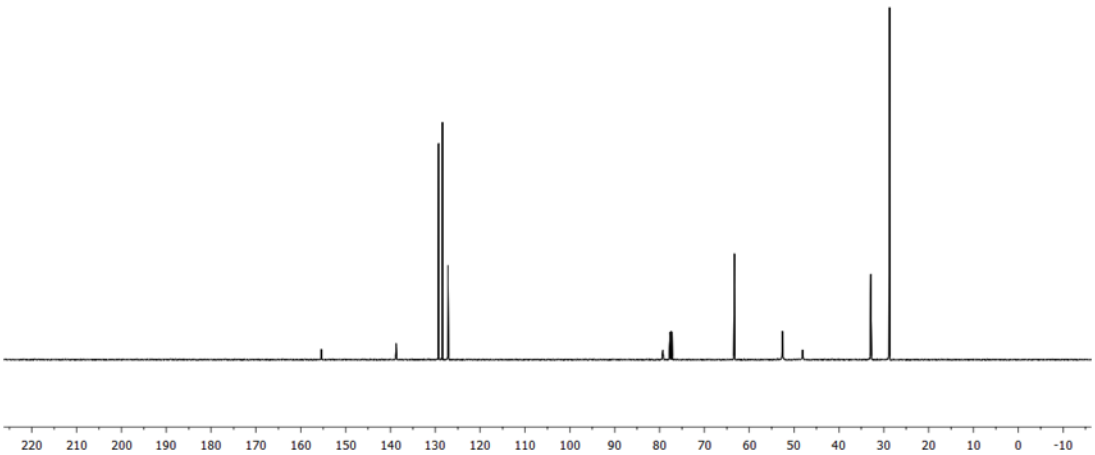
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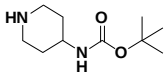
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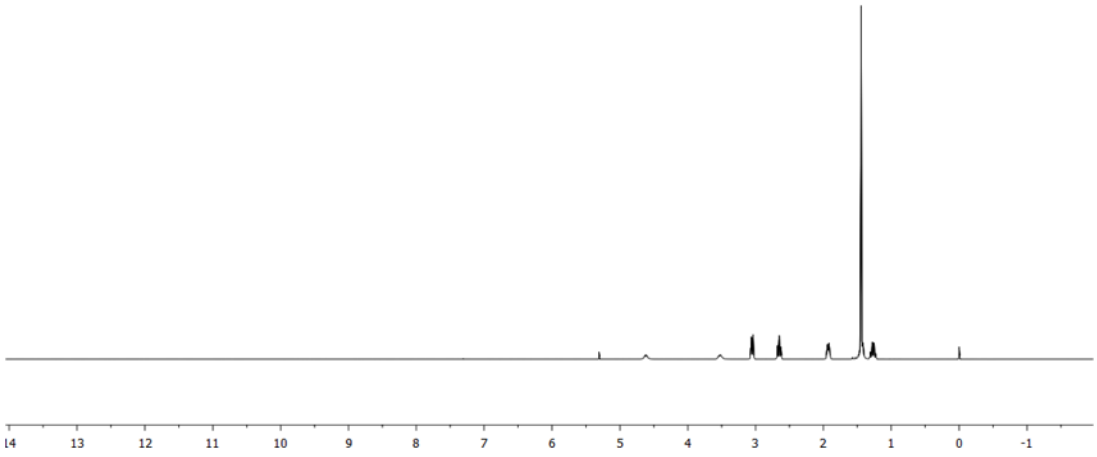
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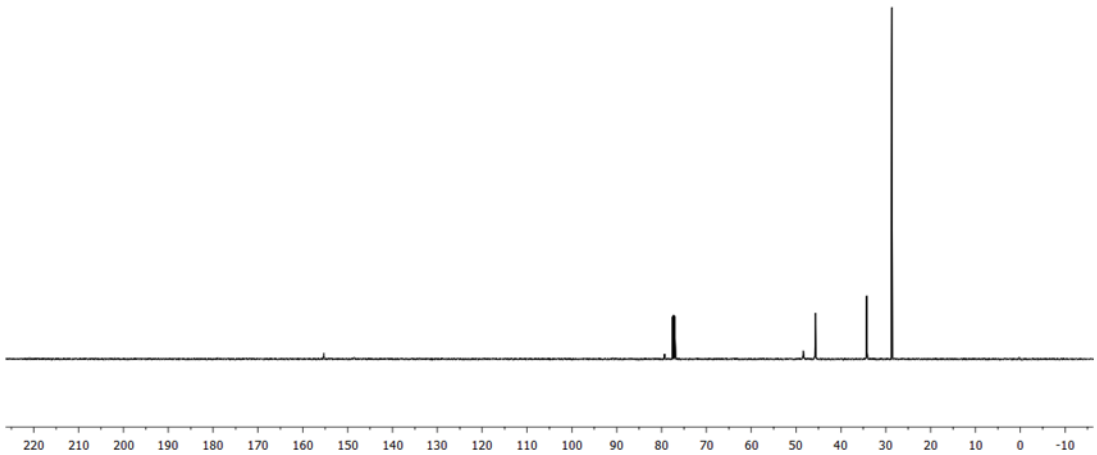
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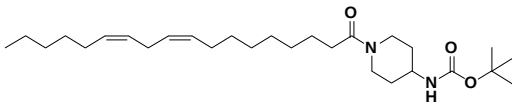

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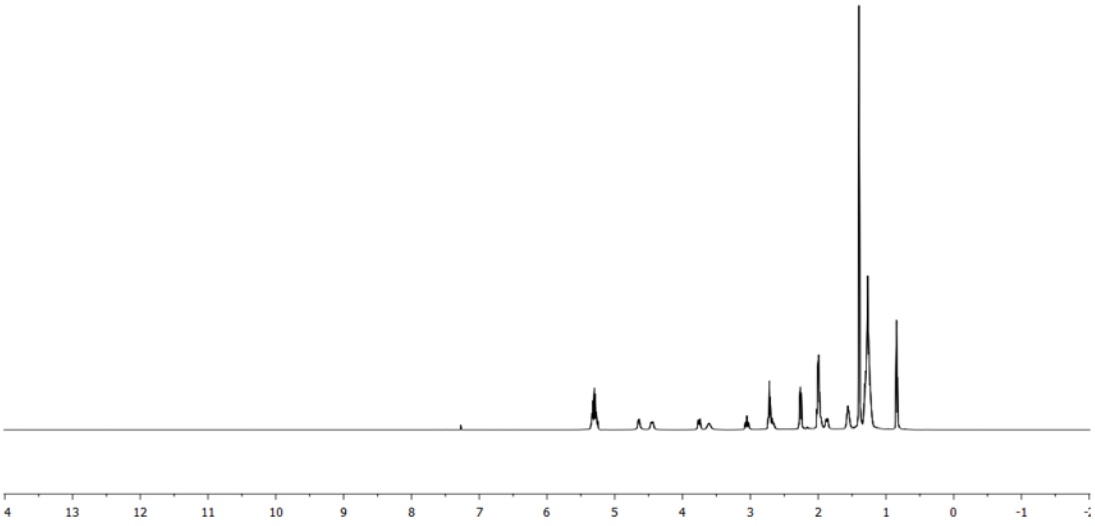
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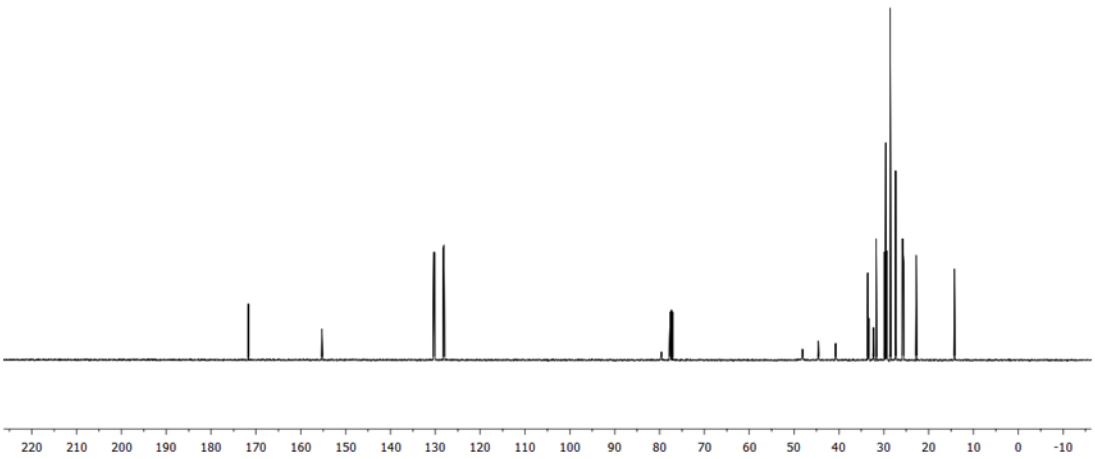
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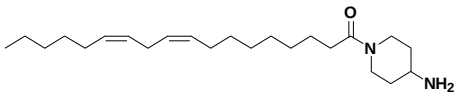
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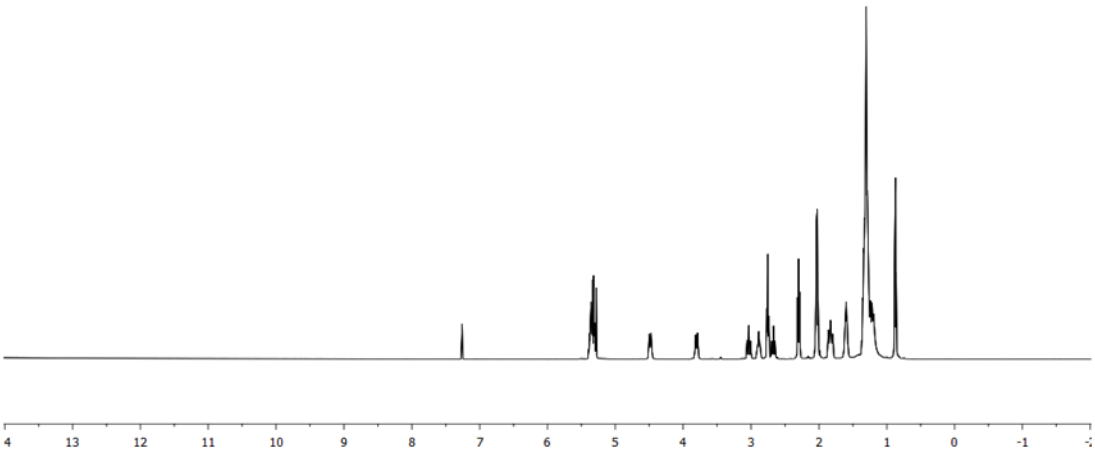
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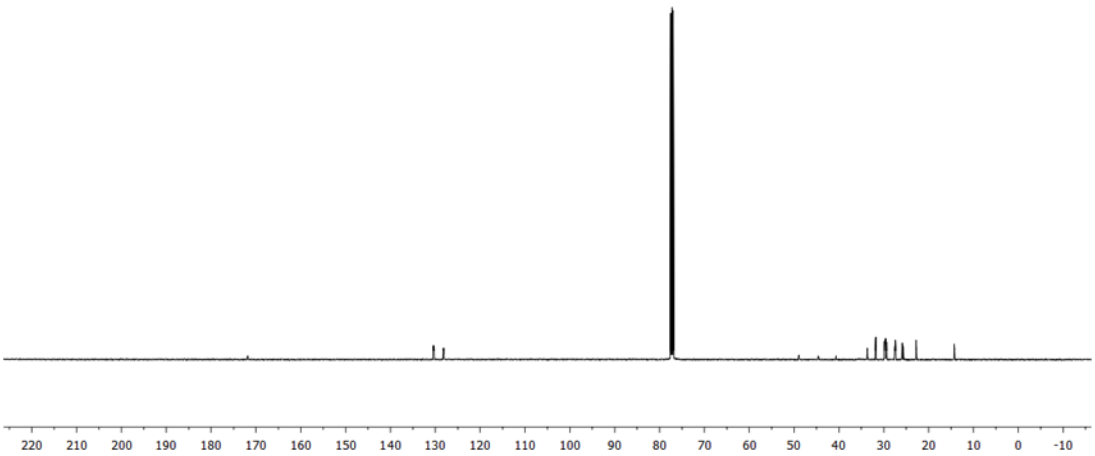
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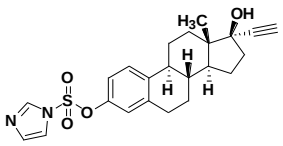
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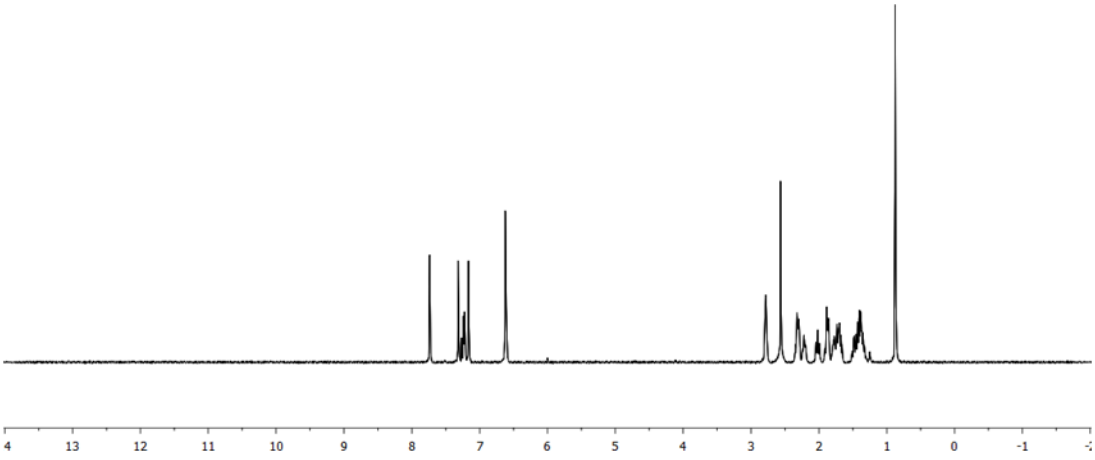
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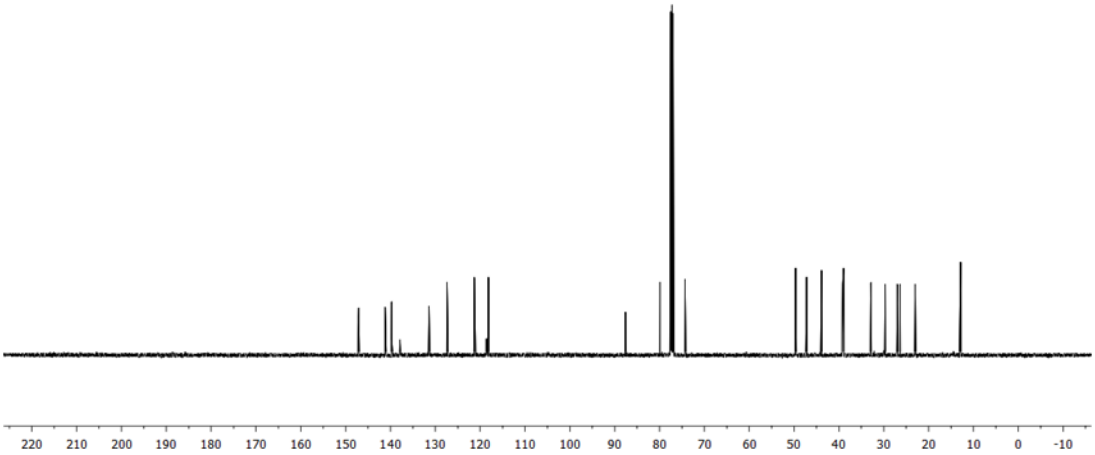
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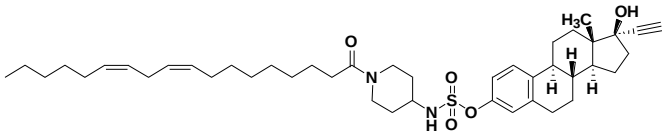
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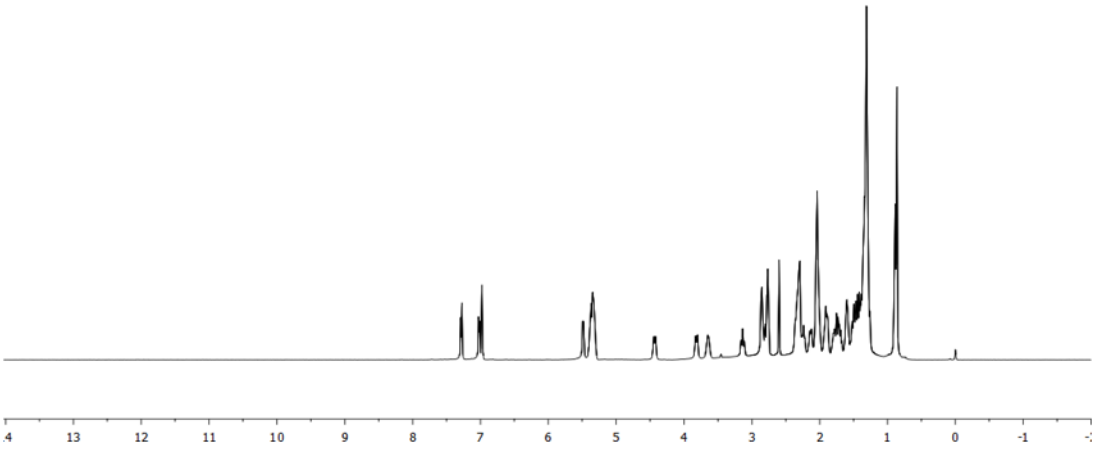
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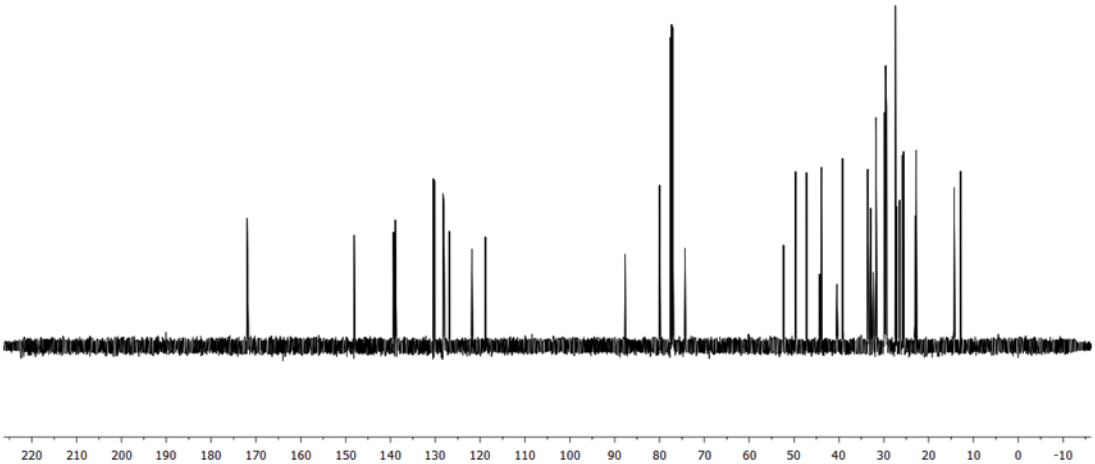
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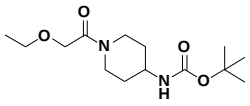

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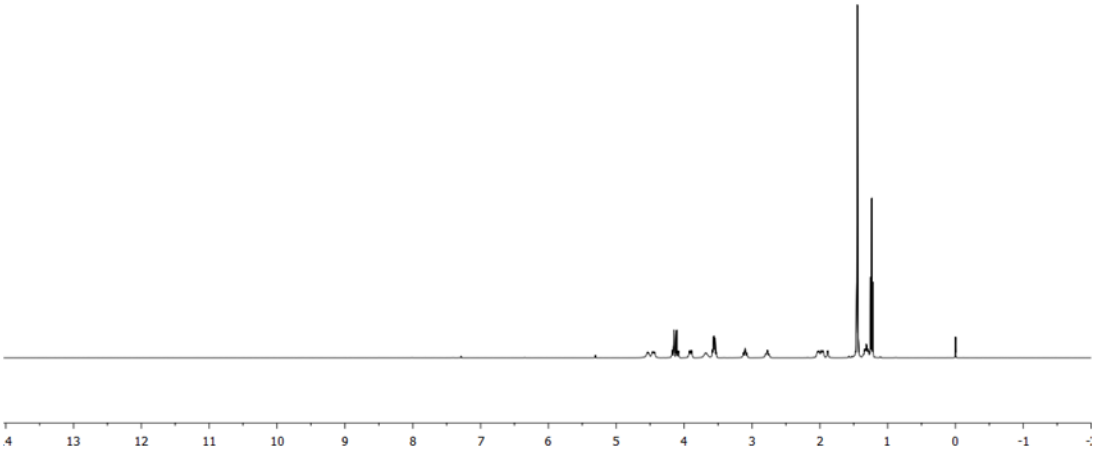

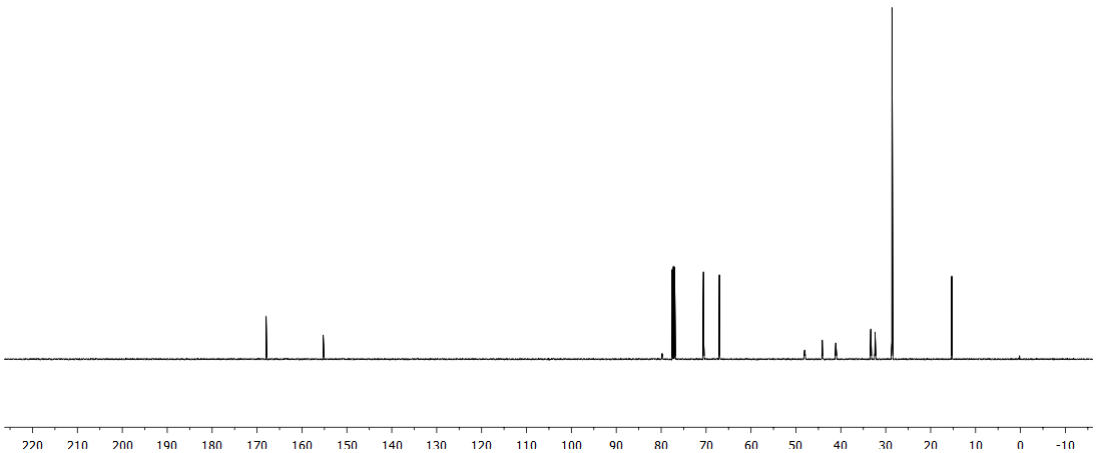

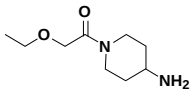
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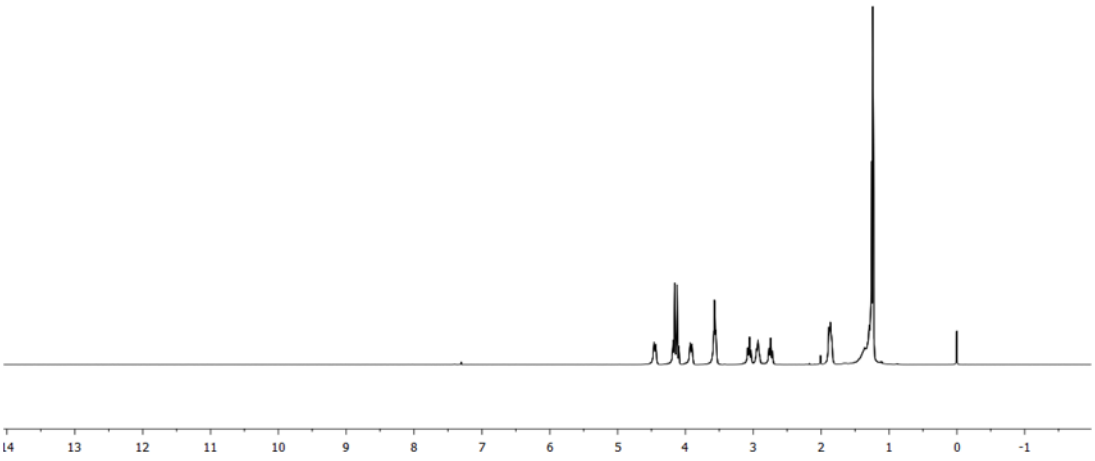
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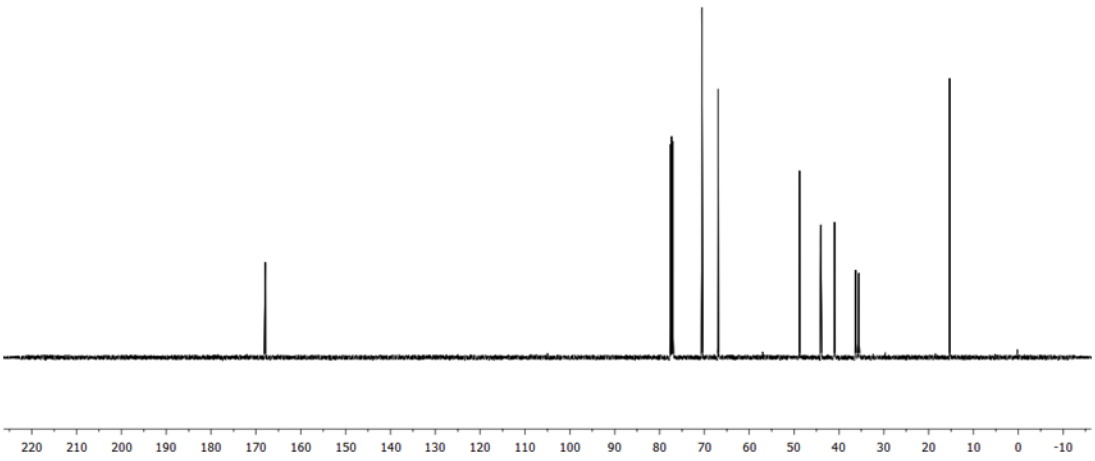
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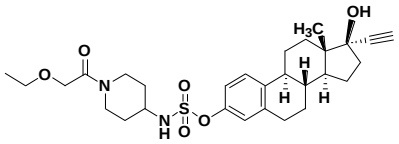
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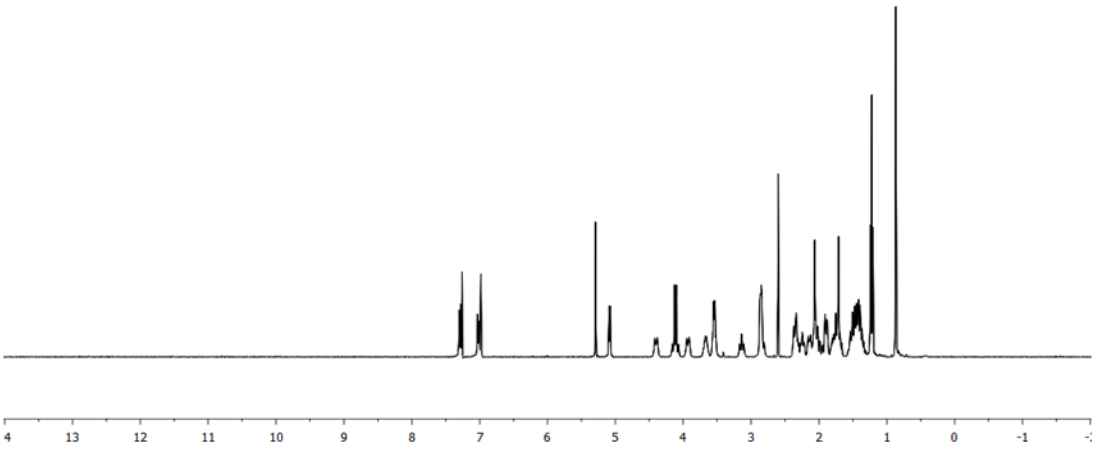
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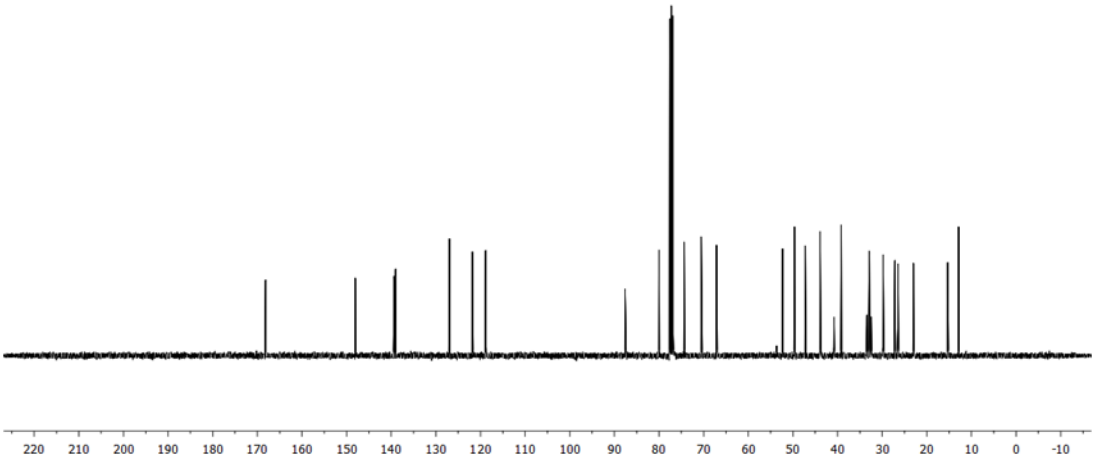
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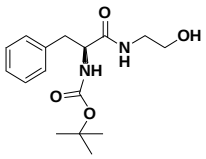
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OH
NH
O
O
O

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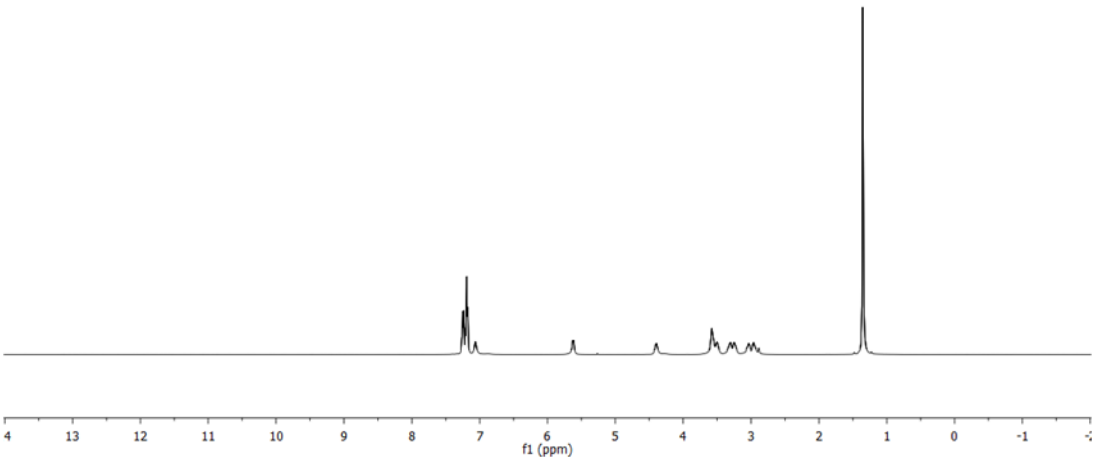
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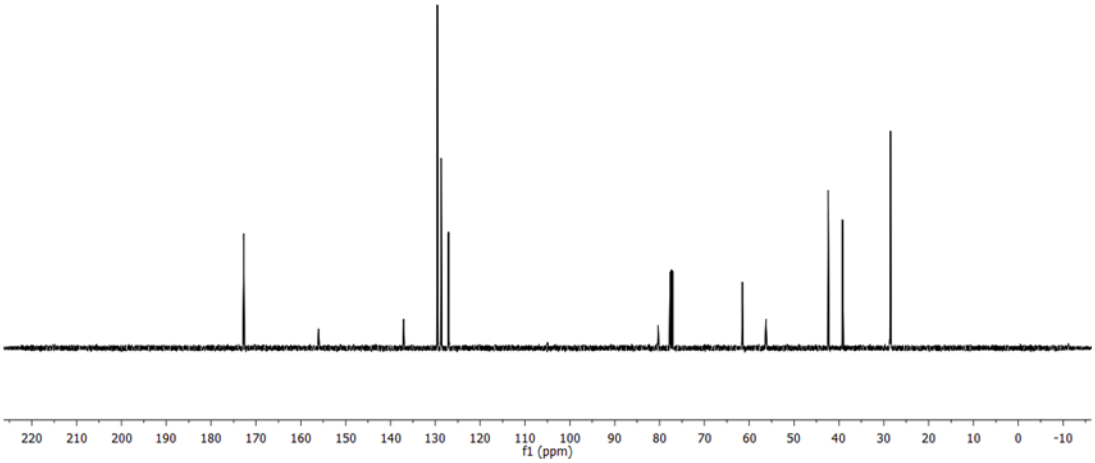
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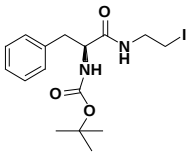

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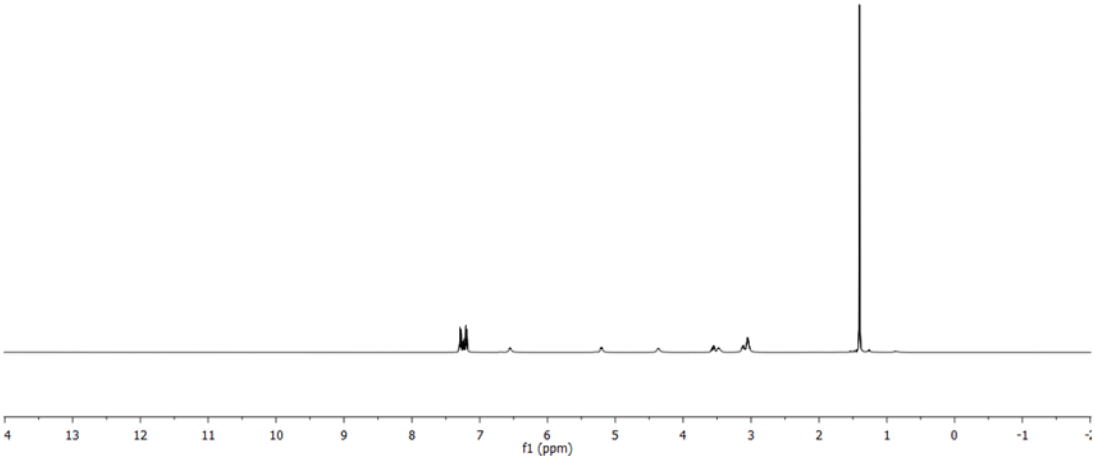

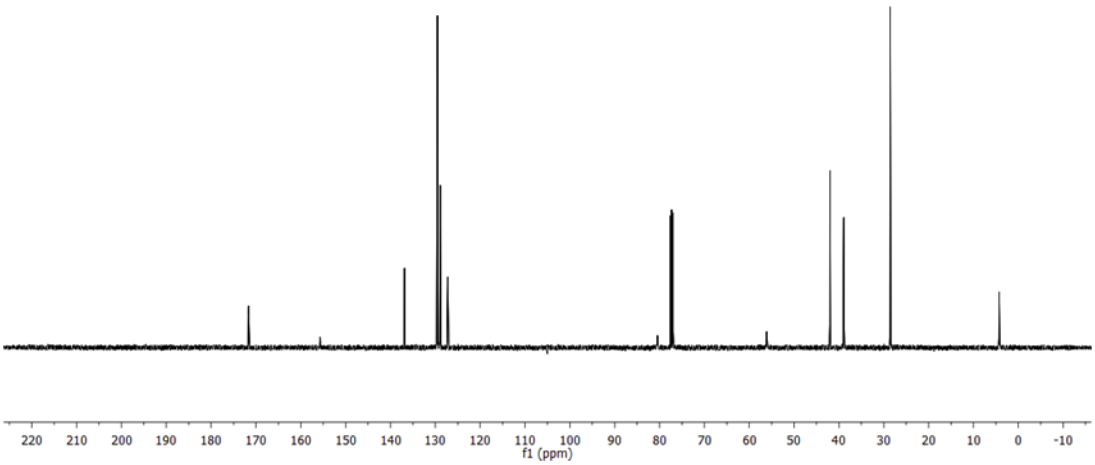

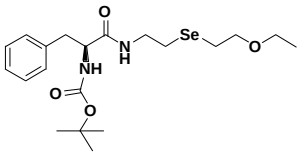
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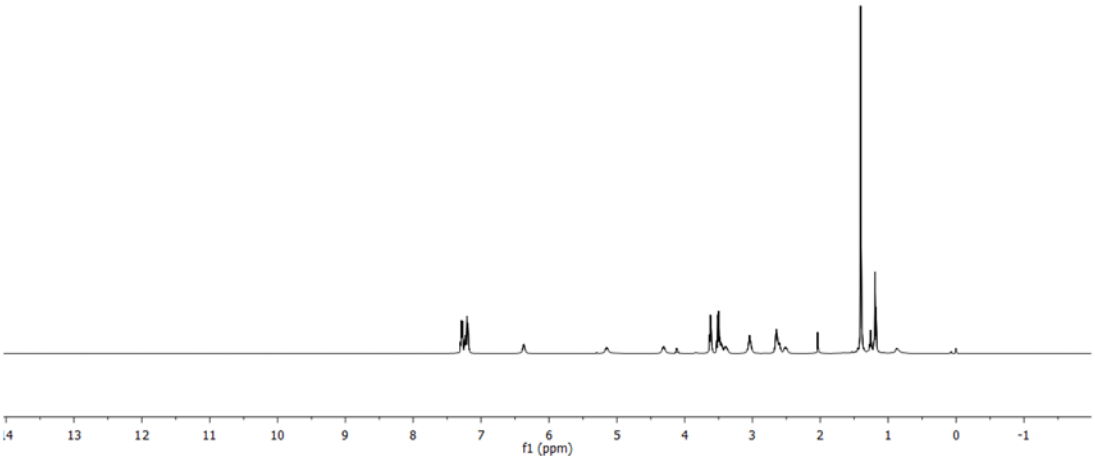
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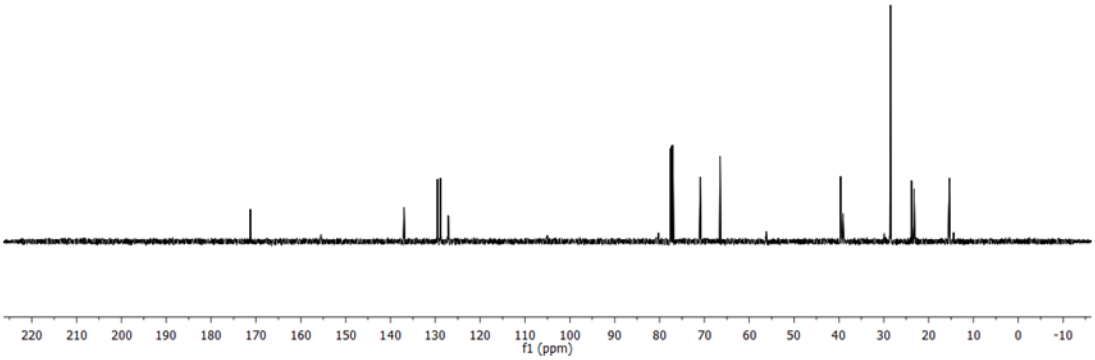
f1 (ppm)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100
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30
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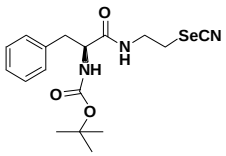
0
-10
f1 (ppm)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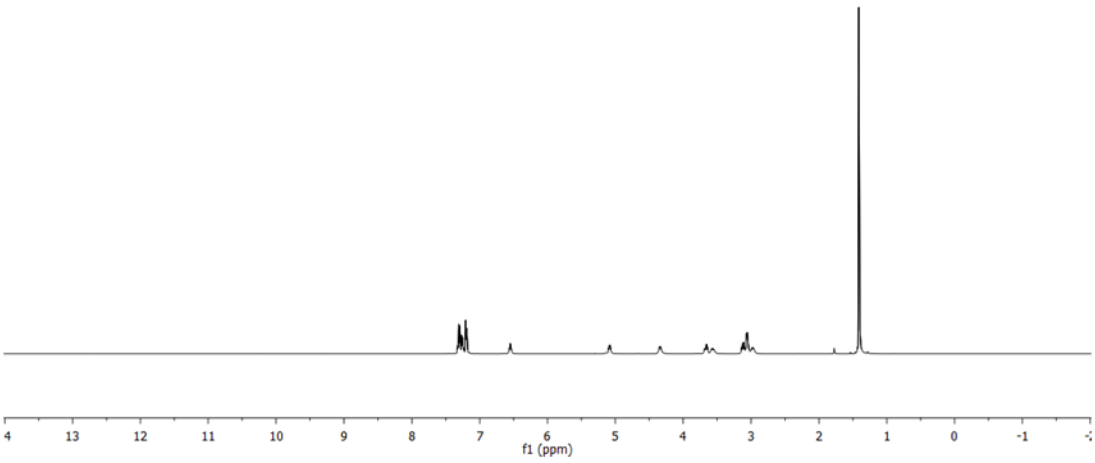
SeCN
NH
O
O
O

2-5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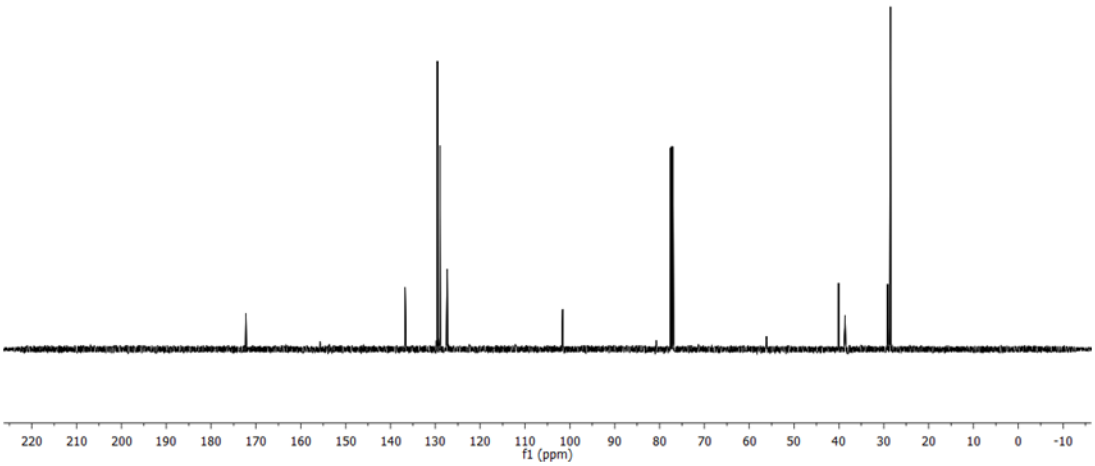
3
2
1
0
-1
f1 (ppm)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100
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60
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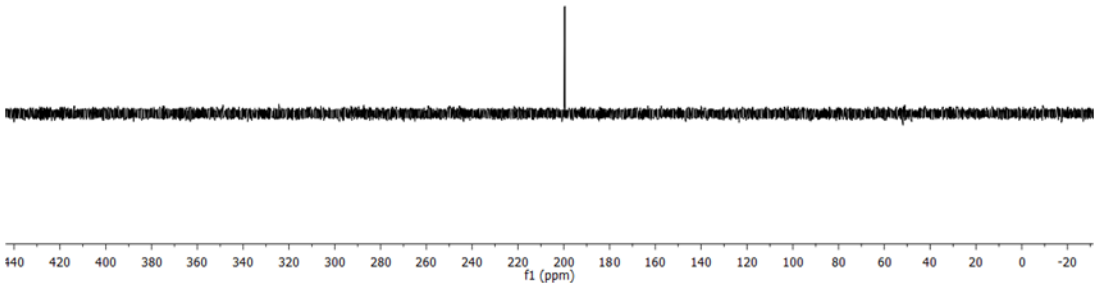
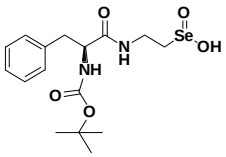
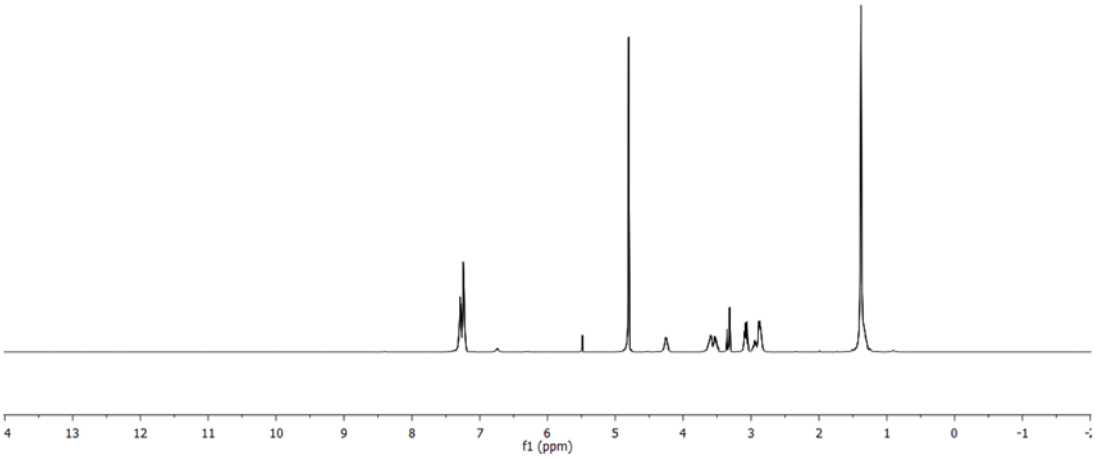
40
30
20
10
0
-10
f1 (ppm)


440 420 400 380 360 340 320 300 280 260 240 220 200 180 160 140 120 100 80 60 40 20 0 -20
f1 (ppm)


O
O
N
H
Se
OH
NH
O
O

2-6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10
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8
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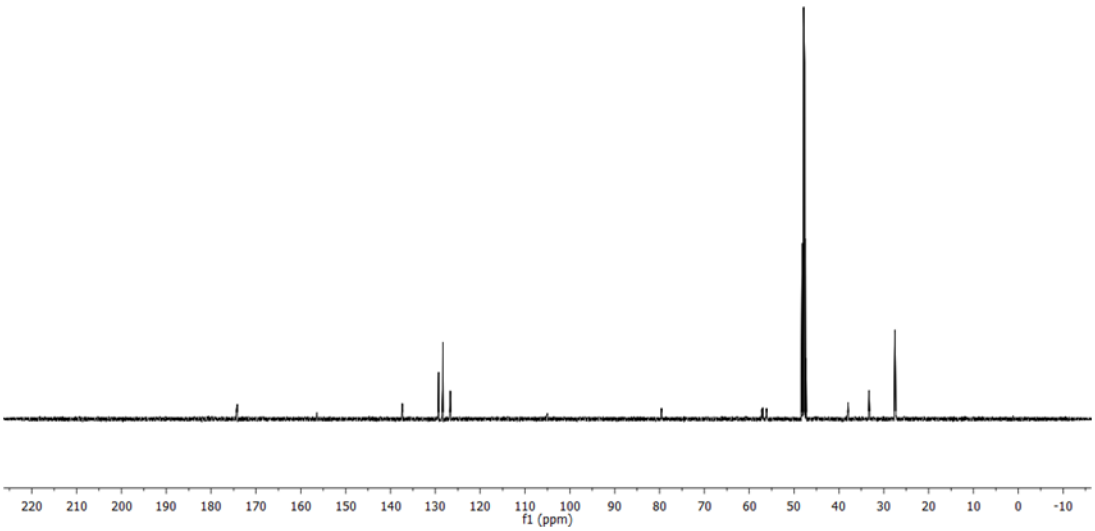
6
5
4
3
2
1
0
-1
f1 (ppm)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180
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120
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100
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80
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60
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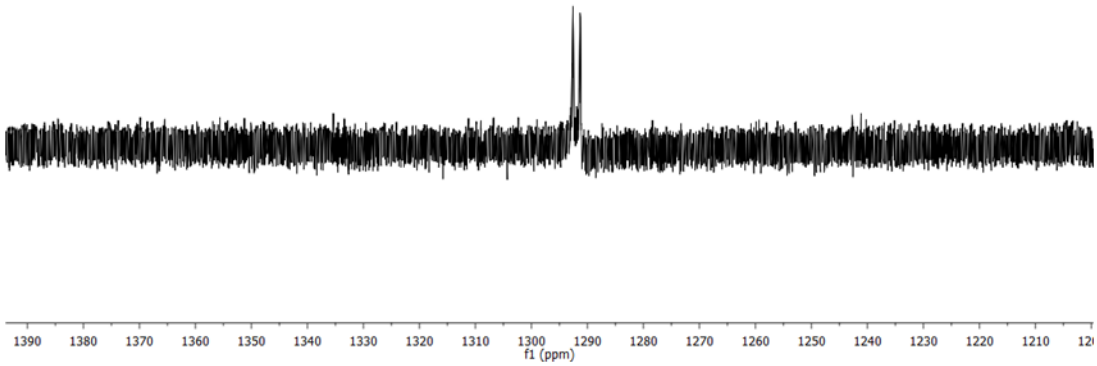
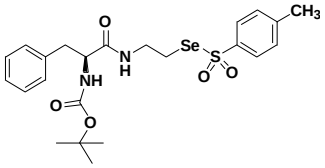
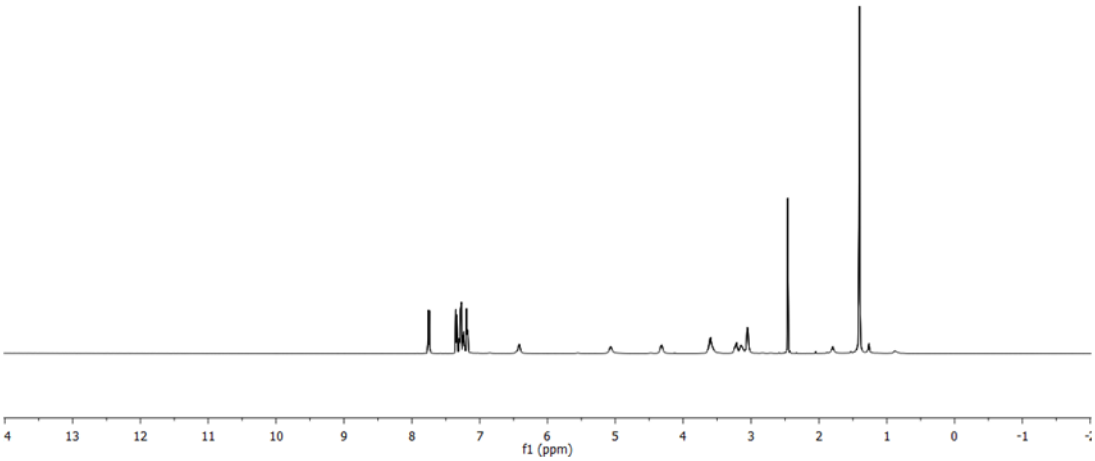
40
30
20
10
0
-10
f1 (ppm)


1390
1380
1370
1360
1350
1340
1330
1320
1310
1300
1290
1280
1270
1260
1250
1240
1230
1220
1210
f1 (ppm)


O
N
H
Se
S
O
O
CH3
NH
O
O

2-7


4
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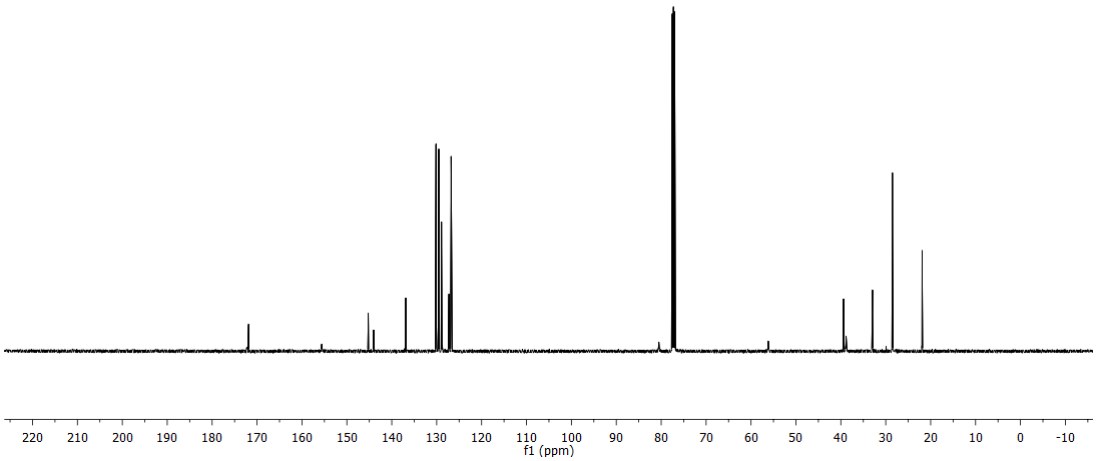
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)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210
200
190
180
170
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120
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100
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80
70
60
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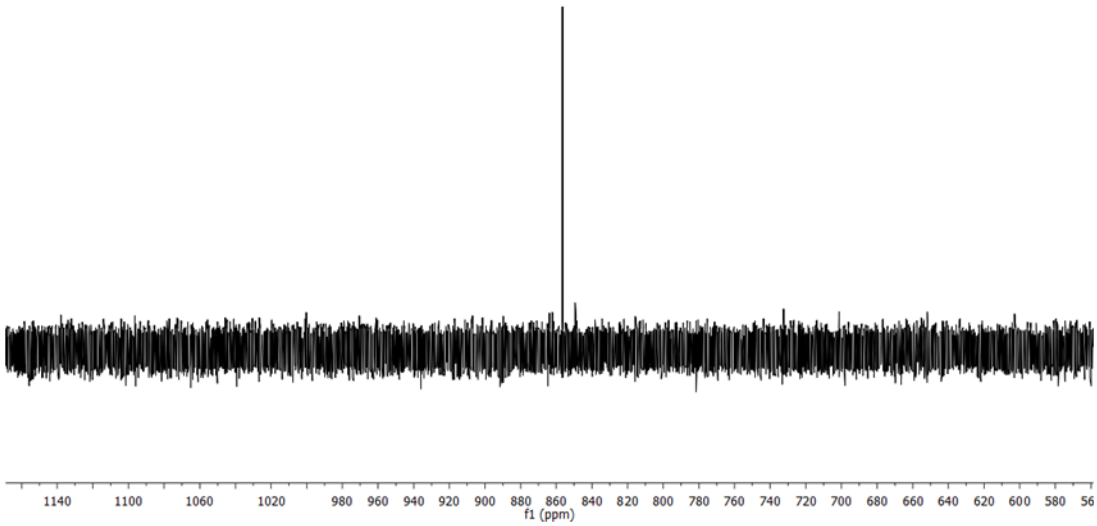
40
30
20
10
0
-10
f1 (ppm)


1140
1100
1060
1020
980
960
940
920
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880
860
840
820
800
780
760
740
720
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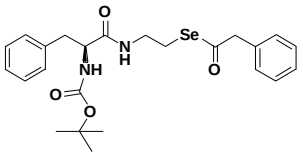
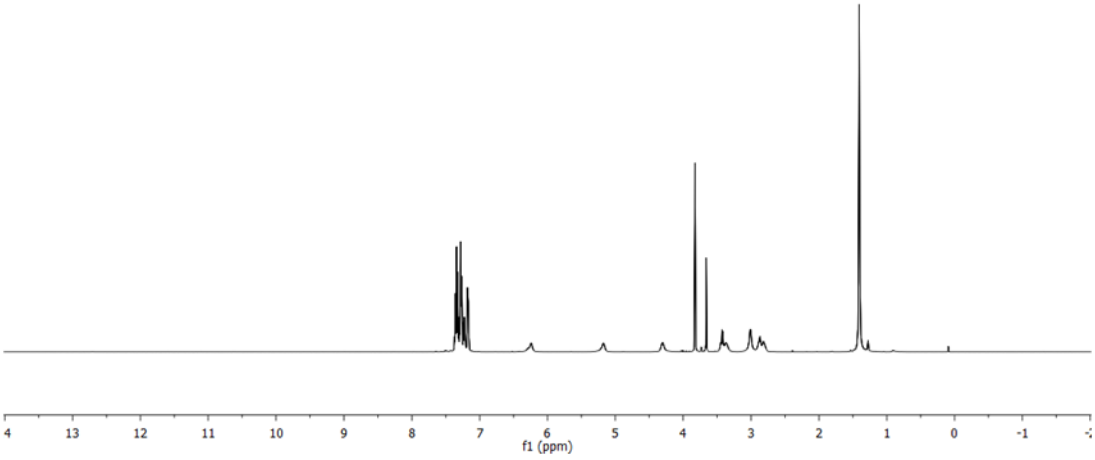
680
660
640
620
600
580
56
f1 (ppm)


O
N
H
Se
O
NH
O
O

2-8


4
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12
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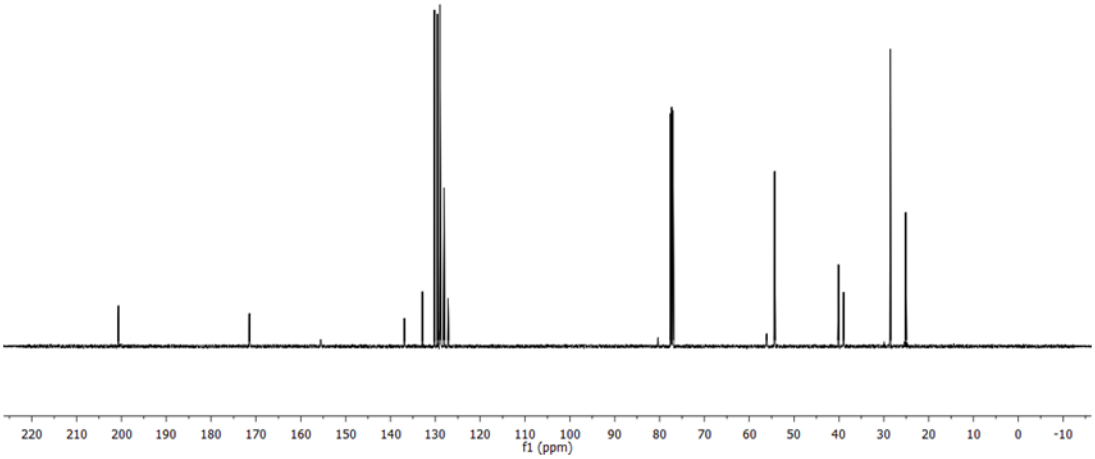
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)


220
210
200
190
180
170
160
150
140
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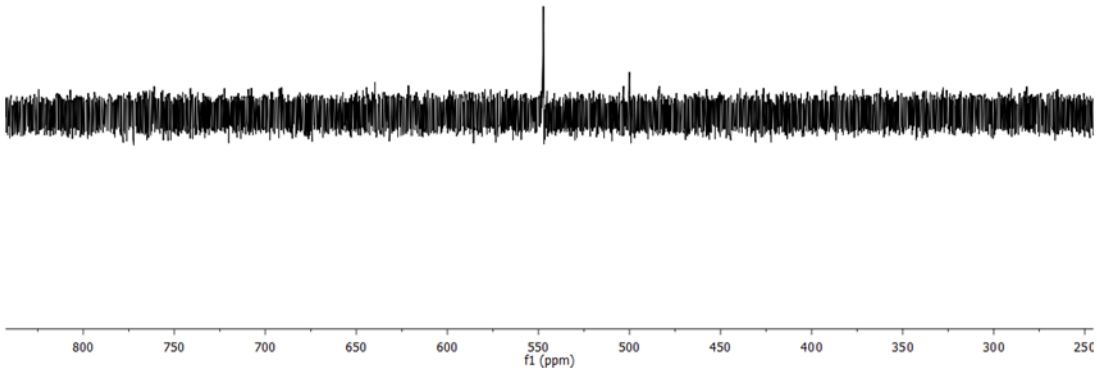
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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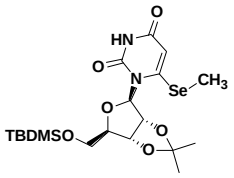
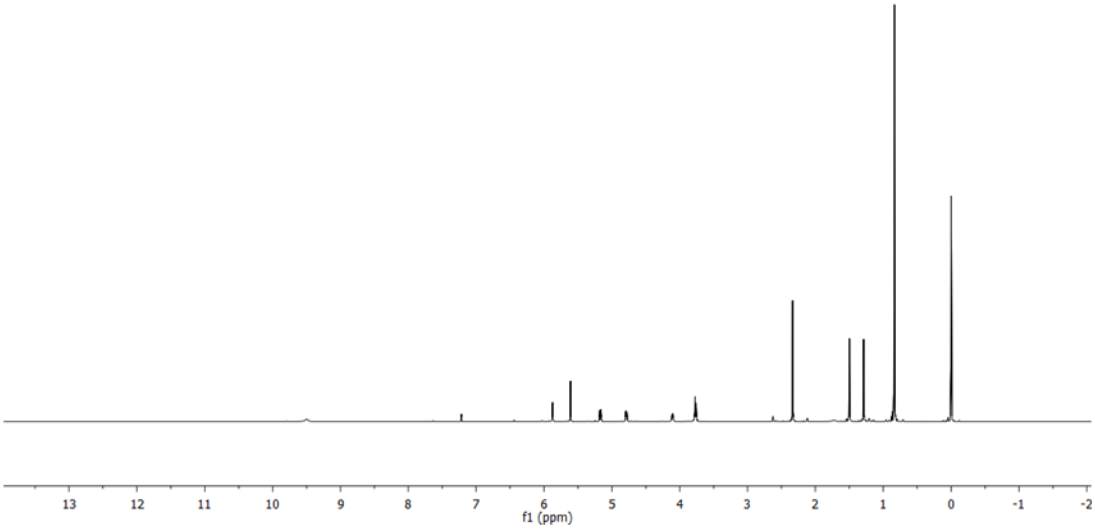
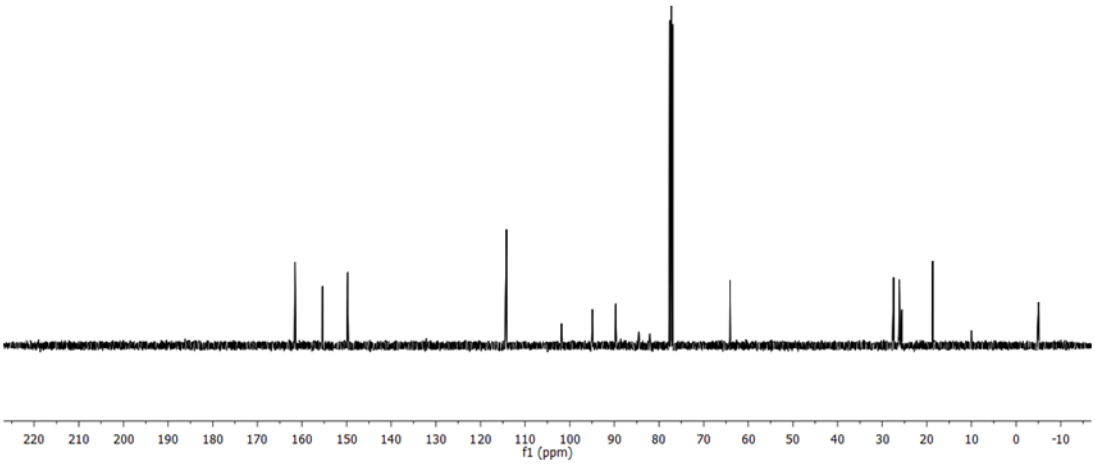
750
700
650
600
550
500
450
400
350
300
f1 (ppm)


O
HN
O
N
Se
CH3
O
TBDMSO
O
O

3-4


13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)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210
200
190
180
170
160
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140
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120
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100
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80
70
60
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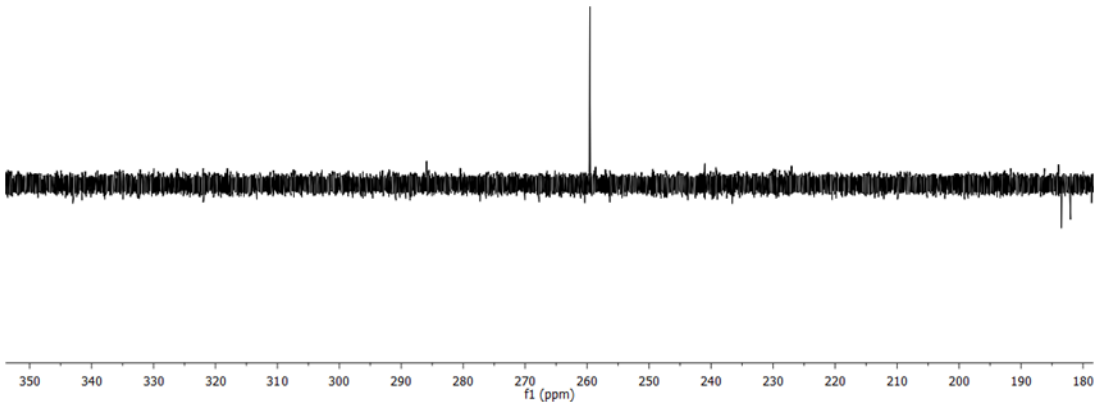
40
30
20
10
0
-10
f1 (ppm)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310
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270
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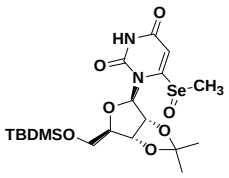
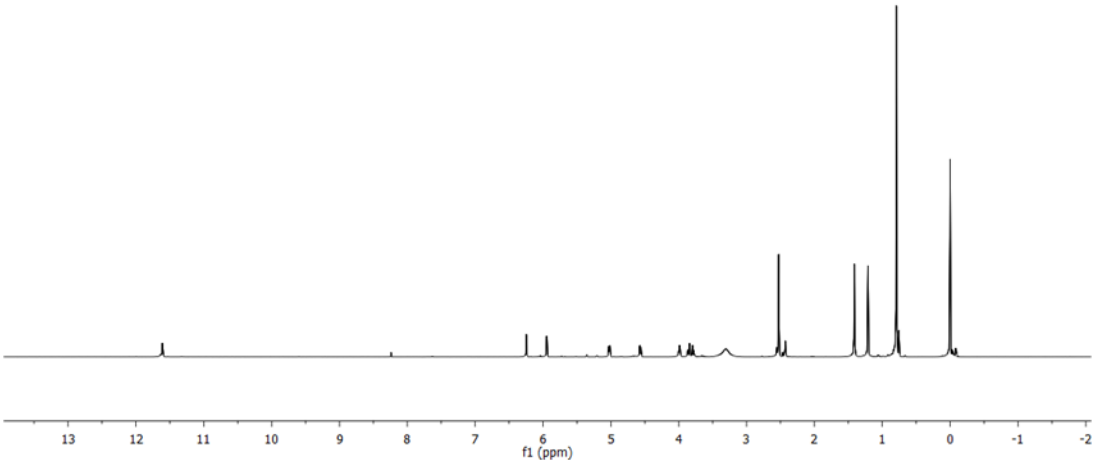
250
240
230
220
210
200
190
180
f1 (ppm)


HN
O
O
N
Se
CH3
O
O
O
O
TBDMSO

3-5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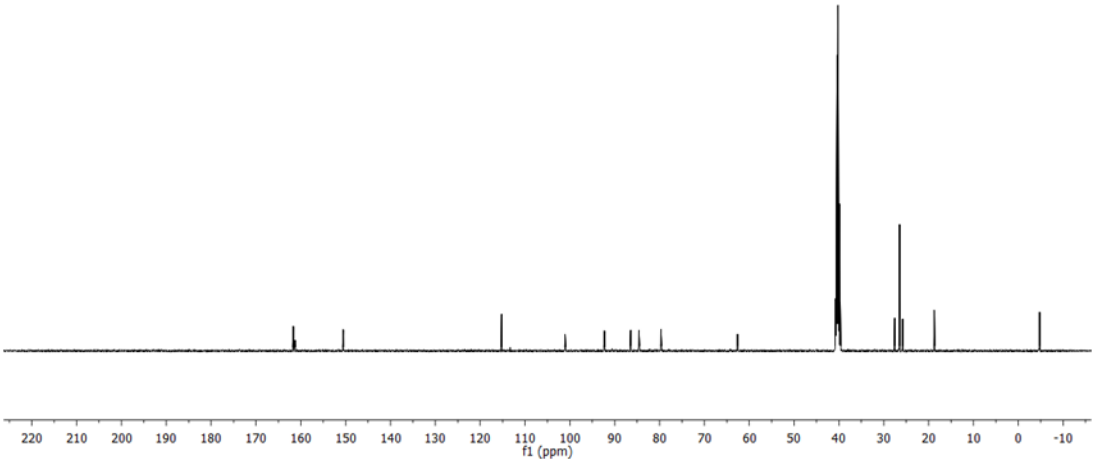
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)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180
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100
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60
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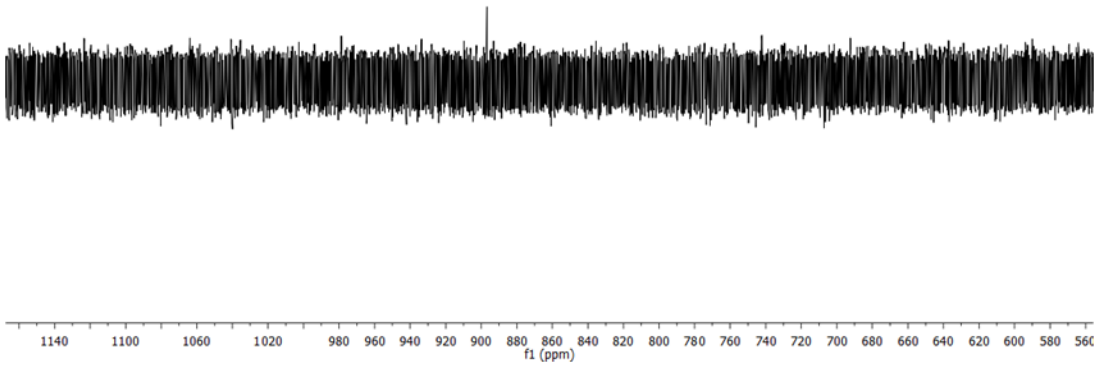
0
-10
f1 (ppm)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1100
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760
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720
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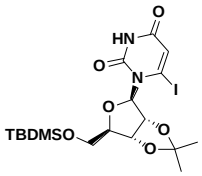
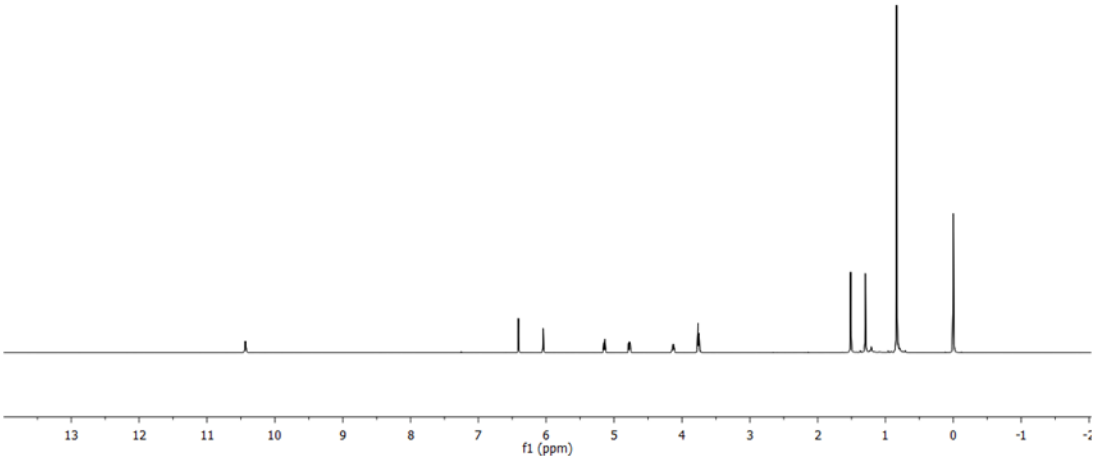
680
660
640
620
600
580
f1 (ppm)


O
HN
O
N
I
O
TBDMSO
O
O

3-6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8
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6
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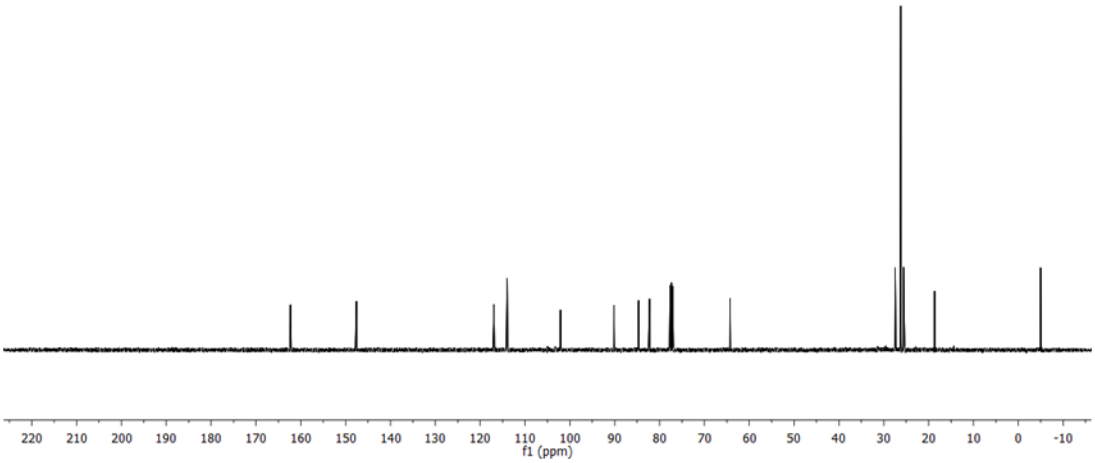
4
3
2
1
0
-1
f1 (ppm)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30
20
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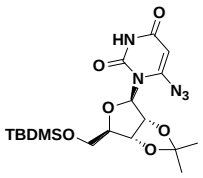
0
-10
f1 (ppm)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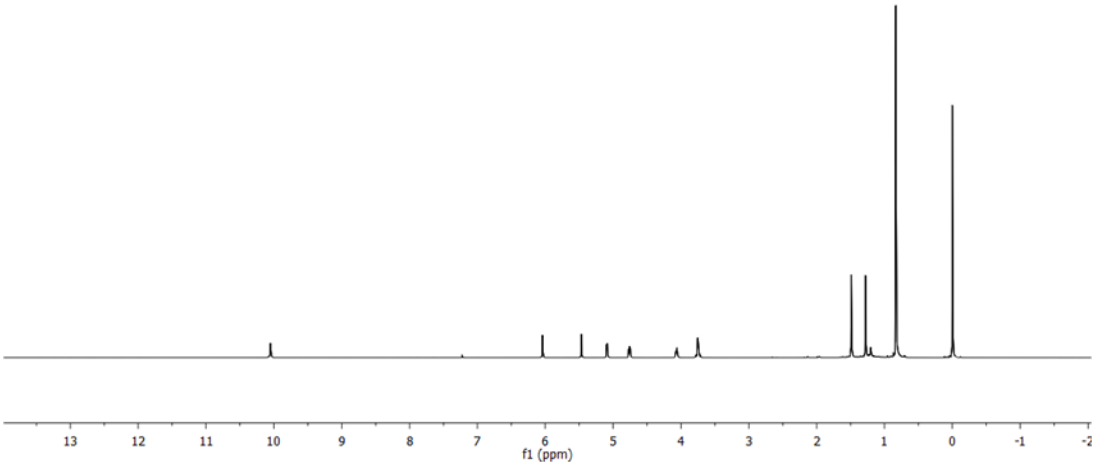
O
N
N3
O
TBDMSO
O
O

3-7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6
5
4
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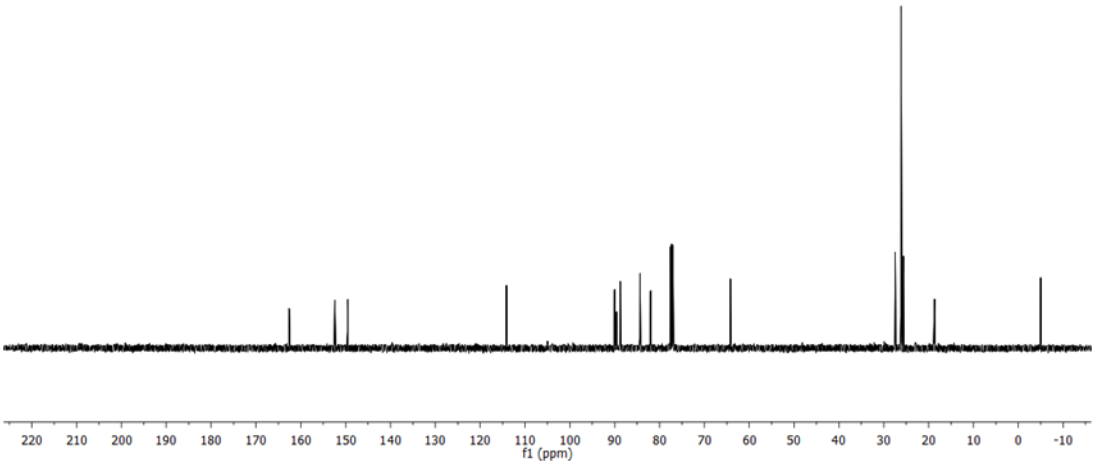
2
1
0
-1
f1 (ppm)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20
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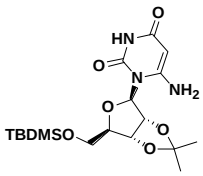
0
-10
f1 (ppm)


O
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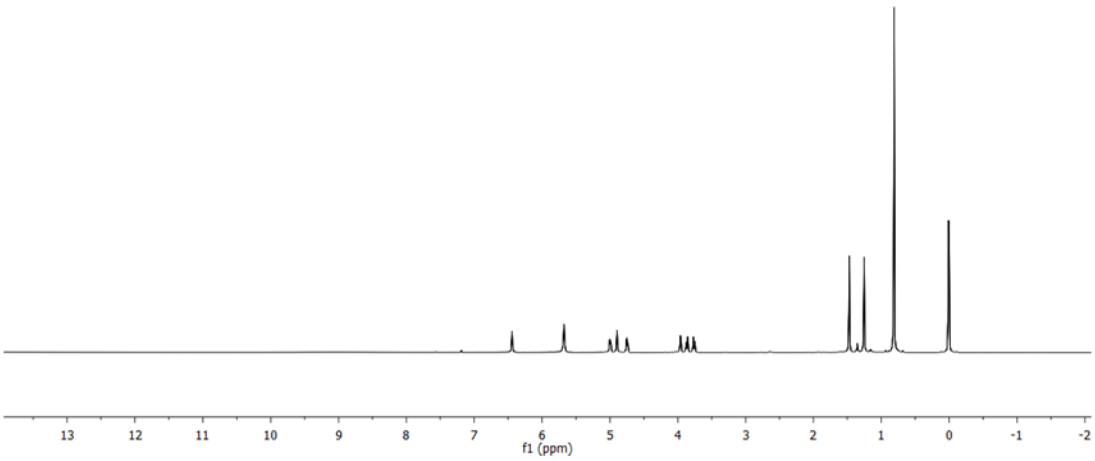
O
N
NH2
O
TBDMSO
O
O

3-8


13
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10
9
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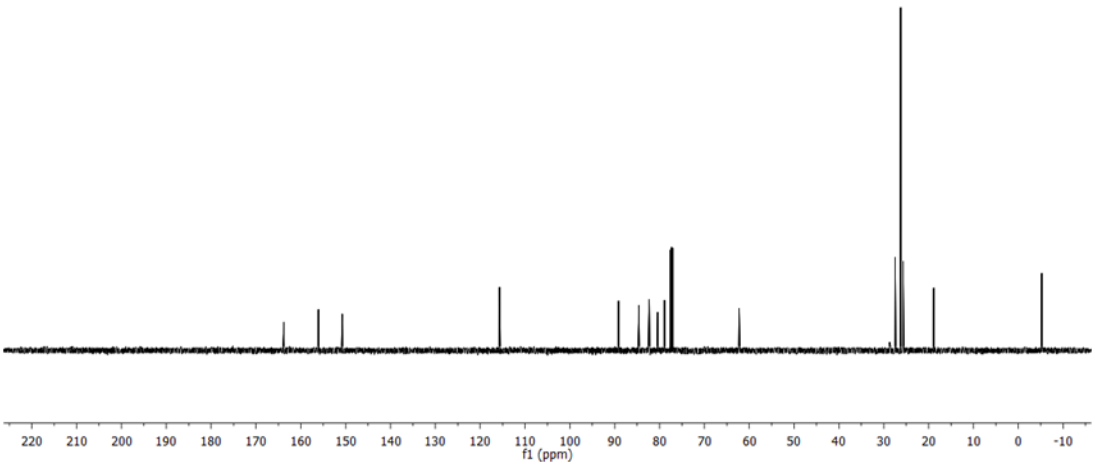
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)


220
210
200
190
180
170
160
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140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)